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PB80-111800

Safety Evaluation of Renovated Wastewater from a Poultry Processing Plant

Pittsburgh Univ, PA Graduate School of Public Health

Prepared for

Environmental Protection Agency, Cincinnati, OH

Aug 79

United States
Environmental Protection
Agency

Cincinnati OH 45268

EPA-600/1-79-030
August 1979

Research and Development

PB80-111800

EPA

Safety Evaluation of Renovated Wastewater from a Poultry Processing Plant

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-79-030		PB80-111800

4. TITLE AND SUBTITLE
Safety Evaluation of Renovated Wastewater from a Poultry Processing Plant

5. REPORT DATE
August 1979 issuing date

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
J. B. Andelman

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Pittsburgh
Graduate School of Public Health
Pittsburgh, Pennsylvania 15261

10. PROGRAM ELEMENT NO.
1BA607

11. CONTRACT/GRANT NO.
R804286 & S803325

12. SPONSORING AGENCY NAME AND ADDRESS
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

13. TYPE OF REPORT AND PERIOD COVERED
Final Aug. 1974-Dec. 1977

14. SPONSORING AGENCY CODE
EPA/600/10 & EPA/600/12

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A three-phase evaluation of reclaimed process wastewater for reuse was undertaken at the Sterling Processing Corporation plant in Oakland, Maryland. The main objective was to evaluate the safety for human consumption of poultry exposed during processing to an average 50 percent mixture of treated well water and reclaimed wastewater. To that end, a determination was made of the ability and reliability of the water reclamation system to deliver satisfactory quality water, and whether the processed poultry would have any excess microbiological or chemical constituents, harmful to human health, as a result of exposure to such water. After the renovation system was optimized (Phase 1), a two-month study (Phase 2) was instituted, which simulated recycle of renovated water through the poultry plant. Chemical, physical, and microbiological analyses were performed on various water, wastewater and poultry samples. An experimental chiller, filled with renovated water, was utilized to compare the uptake of such constituents by the processed birds with that resulting from exposure to the chiller in the processing plant using the normally treated well water. An evaluation of the Phase 2 study, as well as other data, leads to the conclusion that the safety of the consumers of the poultry would not be jeopardized if the planned trial period of reuse (Phase 3) were instituted.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Public health, microbiology, water treatment, potable water, water quality, water reclamation, industrial wastes, industrial water, industrial waste treatment, poultry, ground water, organic wastes	Advanced waste treatment, industrial reuse, renovated water, drinking water standards	57 U

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to the public	Unclassified	90
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	A05-A01

EPA Form 2220-1 (Rev. 4-77)

U.S. GOVERNMENT PRINTING OFFICE 1979-657-060/5417

EPA-600/1-79-030
August 1979

SAFETY EVALUATION OF RENOVATED WASTEWATER FROM A POULTRY PROCESSING PLANT

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. To that end, the Industrial Environmental Research Laboratory assists in developing and demonstrating new and improved methodologies that will provide more efficient and economical pollution control methods. Considerable effort is exerted in developing industrial waste recycle systems which will reduce pollution and conserve our natural resources. The primary mission of the Health Effects Research Laboratory is to provide a sound health effects data base in support of the regulatory activities of the U.S. Environmental Protection Agency. HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes a joint research effort by the two Laboratories. A system was evaluated whereby a poultry processing plant could conserve water and reduce stream pollution without detectable adverse effects on the poultry product.

David G. Stephan
Director
Industrial Environmental
Research Laboratory

R. John Garner
Director
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PREFACE

Wastewater reuse is an important strategy in achieving the national goal of limiting discharges into navigable waters. However, in such reuse of water involving human consumption or exposure, such as in the food processing industry, it is mandatory that the health of the consumer be protected. At the same time it is necessary for both the public and the appropriate professions to adjust their views and judgments on both the esthetic and technical aspects of such reuse. For example, the U.S. Department of Agriculture requires that water used in most phases of food processing be potable. Traditional standards of potability, currently being revised, may not be sufficient to deal with the possible chemical modifications and build-up of concentrations of these materials that can occur in recycle, particularly those for which health criteria may be unavailable.

The reuse of wastewater, involving potential risk from human exposure, has been practiced in agriculture, such as in irrigation, the impoundment of water for recreational purposes, groundwater recharge, and even for domestic water supplies. The most notable example of the latter is Windhoek, South Africa, at which sewage was reclaimed for direct potable use starting in 1968. No such reuse of wastewater for potable purposes is, however, currently being practiced in the United States.

The water reuse project reported here was designed to provide a safe and economical supplemental supply of water at and to a poultry processing plant, utilizing as its base an existing wastewater treatment system. Although not intended for direct human consumption, the renovated water, prior to its use in the poultry processing plant, must meet the highest standards of safety. Because of the general lack of experience in practicing and evaluating such water reuse, both existing standards of water quality and, probably even more importantly, professional judgment are required to assure that this important criterion is met.

ABSTRACT

A three-phase evaluation of reclaimed process wastewater for reuse was undertaken at the Sterling Processing Corporation plant in Oakland, Maryland. The main objective was to evaluate the safety for human consumption of poultry exposed during processing to an average 50 percent mixture of treated well water and reclaimed wastewater. To that end, a determination was made of the ability and reliability of the water reclamation system to deliver satisfactory quality water, and whether the processed poultry would have any excess microbiological or chemical constituents, harmful to human health, as a result of exposure to such water. After the renovation system was optimized (Phase 1), a two-month study (Phase 2) was instituted, which simulated recycle of renovated water through the poultry plant. Chemical, physical, and microbiological analyses were performed on various water, wastewater and poultry samples. An experimental chiller, filled with renovated water, was utilized to compare the uptake of such constituents by the processed birds with that resulting from exposure to the chiller in the processing plant using the normally treated well water.

With only a few exceptions, the mean and even maximum concentrations of the various measured constituents met existing U.S. standards for potable water. In the rare cases when they did not, such as a maximum value marginally exceeding a recommended limit for sulfate, even with direct consumption of the water there would be no danger to human health. There were increased concentrations of several chemical parameters compared to those in the normally-treated well water. However, this is to be expected in a recycle system, and the levels would not jeopardize the health and safety of the consumers of the poultry in actual reuse. The gross organic concentrations in the well water supply and the renovated water were high, but no harmful concentrations of specific organic chemicals were found to be present. For all comparable chemical constituents analyzed in the poultry exposed to the two types of water, the mean concentrations of carcass washings were statistically indistinguishable. Also, it was determined that in most cases there was a net leaching of chemicals from the carcasses to the chiller water, rather than vice versa. An evaluation of the Phase 2 study, as well as other data, leads to the conclusion that the safety of the consumers of the poultry would not be jeopardized if the planned trial period of reuse (Phase 3) were instituted.

This report was submitted in fulfillment of Grant Nos. R-804286 and S-803325 by the University of Pittsburgh and the Maryland Department of Health and Mental Hygiene under sponsorship of the U.S. Environmental Protection Agency. This report covers the period August 1974 to December 1977, and work was completed as of January 1979.

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ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

BGA	--brilliant green agar	PFU	--plaque forming units
CCE	--carbon chloroform extract	SBG	--selenite brilliant green
MPN	--most probable number	TET	--tetrathionate
NDV	--Newcastle Disease Virus	TOC	--total organic carbon

SYMBOLS

Sampling Points

A	Raw wastewater	L-1	Lagoon 1
C	Lagoon system effluent, chlorinated	L-1-M	Lagoon 1, middle
		L-1-E	Lagoon 1, effluent
C'	Lagoon system effluent, unchlorinated	L-2	Lagoon 2
		PB	Carcasses from plant chiller
D	Micro-strainer effluent		
DS	River, downstream	PC	Water from plant chiller
E	Renovated water	R	River, downstream
EB	Carcasses from exptl. chiller	US	River, upstream
		X	Effluent, sediment-basin
EC	Water from exptl. chiller	Y	Well water, untreated
		Z	Well water, treated

Miscellaneous

b	Fraction remaining in lagoons	C_x	Conc. at x sample point
		N	Number of samples in statistical calc.
c	Fraction remaining in renov. system		
C_n	Conc. in 50/50 renov. mixture after n cycles	r_s	Ratio of C_s to C_E
		S.D.	Standard deviation
C_s	Conc. in 50/50 renov. mixture in steady-state	$\bar{X}$	Mean concentration

ACKNOWLEDGMENT

This study was a joint effort by the Maryland Department of Health and Mental Hygiene (MHD), the Sterling Processing Company, Oakland, Maryland, and the Graduate School of Public Health, University of Pittsburgh (GSPH). The University of Pittsburgh wishes to acknowledge with thanks the cooperation of the MHD and in particular James Clise, Director, Bureau of Sanitary Engineering and Col. Edward, S. Hopkins, Consulting Sanitary Engineer for the MHD, who initiated and were the principal investigators for Phase 1 of the overall study, funded by US EPA Grant S-803325, during which the water renovation system was modified and optimized. They also prepared the portion of this report concerning the operation of this system. The Cumberland and Central Laboratories of the MHD performed many of the daily chemical and bacteriological analyses for the facility; major responsibility being undertaken by Mary Lynn Hotchkiss and Mary Elizabeth Malloy of the Cumberland Laboratory and Muriel W. Trusheim of the Central Laboratory. Dan McGrail was in charge of plant operation from May 1975 to August 1976, followed by Robert Holtschneider from November 1976 to June 1977, both under the immediate supervision of Col. Hopkins. Thomas Sereno, MHD aide, was responsible for collection of samples submitted to the Cumberland Laboratory.

It is also a pleasure to acknowledge the enthusiastic cooperation and persistence of Gilman Sylvester, the manager of the Sterling Processing Corpporation, as well as his staff. Several analyses, including those for pesticides and a few trace elements, were performed by the Analytical Services Laboratory of the NUS Corporation, Cyrus Wm. Rice Division, Pittsburgh, Pa., under the able direction of Ellen Gonter.

Faculty at the University of Pittsburgh who were particularly helpful were John Armstrong and Robert Yee of the Microbiology Department of GSPH who advised on the virology and bacteriology, respectively; also Iain Campbell of the Biological Sciences Department of the College of Arts and Sciences who advised and otherwise cooperated on the gas chromatography and mass spectrometry analyses.

Teresa Lester and Jan Wachter, members of the research staff of GSPH, made substantial contributions on several aspects of this study, including project planning, operation, data interpretation, and the writing of this report. Other research staff or students working on this project were David Capelli, Robert Lau, Kenneth Wasyczak, John Weaver, and Stephanie Wilson.

Finally, the University of Pittsburgh wishes to acknowledge with appreciation the generous guidance, assistance, and patience of the EPA Project Officers, Herbert Pahren and Jack Witherow.

SECTION 1

INTRODUCTION

In early 1970 a wastewater reclamation project was initiated at a poultry processing plant in Oakland, Maryland located in the far western part of that state near the junction of Maryland, West Virginia, and Pennsylvania. An increase in production at the Sterling Processing Company was and is limited by the lack of additional water of acceptable quality. The plant currently slaughters and processes approximately 50,000 birds per day (usually chickens) in an eight-hour operation, utilizing approximately 1,300,000 liters (350,000 gallons) per day of treated well water.

With substantial financial support, initially from the U.S. Department of Interior in January 1971, and later the U.S. Environmental Protection Agency (EPA), a joint project was developed by the Maryland State Department of Health and Mental Hygiene and the Sterling Processing Corporation to design, construct, and study the feasibility of using a wastewater renovation system, utilizing as its raw water source the chlorinated effluent from the second of two aerated lagoons. These lagoons were initially designed and constructed in 1965-1966 to treat the plant wastewater prior to its discharge into the adjacent Little Youghiogheny River. The goal of this project was to renovate the lagoon wastewater to the point that it could be mixed with the well water, then undergo conventional water treatment and be used in the Sterling plant to process the poultry, thereby providing a supplementary source of water otherwise not available. Since the regulations of the U.S. Department of Agriculture require that the water used to process poultry "shall be ample, clean, and potable," it was deemed important that an extensive study of the quality of the renovated water be instituted prior to its reuse. In a report published by the EPA, Clise describes the initial project, including the construction and operation of the renovation system, and the evaluation of the quality of the renovated water (7).

The full reclamation system, as reported by Clise, begins with the two aerated lagoons, followed by partial diversion of the chlorinated effluent from the second to a microstrainer, then flocculation-sedimentation, chlorination, and filtration. The initial study showed that the reclaimed water met U.S. Public Health Service 1962 Drinking Water Standards for chemical, microbiological and physical constituents without actual recycle through the poultry processing plant. Nevertheless, there was concern that with actual reuse there was the possibility that unmeasured constituents, such as pathogenic microorganisms, heavy metals, pesticides and toxic organic chemicals, might build up in recycle and be absorbed by the carcasses in the processing plant.

An additional project was proposed by the Maryland State Department of Health and Mental Hygiene and funded by the EPA (Grant No. S803325), the purpose of which was to modify and optimize the reclamation system, to determine the capability and reliability of the system for delivering satisfactory water quality, and to evaluate the exposure of the processed carcasses to constituents that could be harmful to human health. With separate funding (Grant No. R804286), EPA supported the Graduate School of Public Health, University of Pittsburgh, to design, supervise, and perform the sampling and analytical part of the study, as well as evaluate the results from the points of view of both the quality of the renovated water and the processed poultry possibly affected by it. Three phases were planned in this study, the first two of which have been completed. Phase 1 involved the operation of the reclamation plant with a new sand filter, and measurement of those characteristics pertinent to optimizing the process. Phase 2 involved a study of a wide range of physical, chemical and microbiological constituents, both at various points in the reclamation system, as well as in process carcasses chilled with renovated water, but without actual recycle through the plant. Phase 3 was to involve recycle of the renovated water into the processing plant by mixing on an average 50/50 basis with well water, the mixture then to undergo additional full-scale conventional treatment. The carcasses were again to be measured, as was the renovated water, and comparisons made of the levels of contaminants with those in normal plant operation utilizing well water only in Phase 2.

Prior to proceeding to Phase 3, an evaluation was to be made of the Phase 2 results by a committee consisting of representatives of the EPA, the Maryland Health Department, the processing plant, the U.S. Department of Agriculture, and the Graduate School of Public Health. The level of contaminants in the renovated wastewater and processed carcasses were to be considered as to their possible health significance. A similar evaluation was to be made following Phase 3 so as to determine the safety of proceeding to continuous operation with reclaimed wastewater. Finally, recommendations were to be made on constituents to be monitored and corresponding procedures needed to insure the reliability of the system and the protection of human health.

This report describes and evaluates primarily the Phase 2 study of the University of Pittsburgh. Additionally, a summary description of the modification and operation of the renovation system under a companion grant by the EPA to the Maryland Department of Health and Mental Hygiene is included.

Phase 3, the trial three-month period of actual recycle of the renovated water into the processing plant, has not been instituted because it has not been approved by the appropriate federal agencies, in spite of the positive recommendation by the above Committee, constituted for the purpose. Some of the likely concerns which are the apparent basis for this lack of approval will also be discussed.

SECTION 2

CONCLUSIONS

Based on the regularly low or zero bacteriological counts in the renovated water during the Phase 2 study, as well as at other times, the absence of any avian virus that could cause human disease by the enteric route, and the extensive wastewater and water treatment, including four points of chlorination, it is highly unlikely that the contemplated reuse of water at the Sterling Processing Corporation plant would pose a risk of disease from microorganisms to the consumers of the poultry.

The inorganic and physico-chemical characteristics of the renovated water consistently met applicable standards of quality for potable water. A few such parameters, and several non-health related ones, as expected were high, but not at levels that would constitute a threat to human health, even if the water were directly ingested. However, in actual reuse it would receive additional treatment and would not be used as a drinking water supply. A few non-health related chemical constituents were at concentrations that could interfere with the optimal operation of the renovations system, and should be adjusted. These include the low pH and high ammonia concentrations.

Some measurements of gross organic load, such as total organic carbon, were high in both the treated well water and the renovated water, but there were no statistical differences between them. None of the specific organic chemicals that were found were at hazardous or unusually high concentrations, and many are innocuous. There is a possible concern that unidentified toxic organics could be present in the renovated water, including those that might form as a result of reactions with chlorine. However, in the measurements that might have uncovered them there was no evidence for their presence.

A comparison of carcasses exposed to the normally-treated well water and to the renovated water in almost every instance showed no statistical difference in the mean concentrations of measured constituents. For a few chemicals, including calcium, ammonia, sulfate, and nitrate, there was an apparent contribution to the carcasses by the chiller water. Calculations indicate that for most of the chemicals there was much more leaching into the chiller water from the carcasses than vice versa.

Mass-balance, steady-state calculations indicate that the Phase 2 recycle simulation had probably reached steady-state, and the concentrations measured in the renovated water could be used as a basis for predicting the levels that would be encountered in actual reuse. For many of the constituents such concentrations would be equal to or as much as one-third less than those found in Phase 2.

An analysis and review of all the measurements and operational characteristics of the renovation system and Phase 2 results indicate that there is no significant or discernible risk to the consumers of the poultry in the planned reuse of the water. It is unlikely that in such reuse any constituent limits for chemical or microorganisms for which there are current potable water quality standards would be exceeded in the renovated water. Also it is unlikely that these or any other constituents, already identified or not, would be taken up by the carcasses in hazardous quantities as a result of exposure to this water.

Low winter temperatures and lagoon turnovers create serious problems for the renovated water system. Its operation should be suspended during these periods.

SECTION 3

RECOMMENDATIONS

The results of this and previous studies of the wastewater and renovation systems at the Sterling Processing Corporation plant indicate that there are not any apparent concentrations of chemicals or microorganisms that did, or in actual reuse would be likely to build up in the renovated water supply to the point of jeopardizing the health of the consumers of the poultry processed with that water. There are some steps that could and should be taken to minimize and further reduce any possible risks or concerns. It is, therefore, recommended:

1. A trial period of reuse (Phase 3) should be instituted as soon as possible. During this period there should be a full scale monitoring of the renovated water and carcasses as originally planned, followed by a comprehensive evaluation process prior to any permanent reuse.

2. Prior to and/or during the trial period of reuse more extensive organic analyses of the renovated water and other pertinent samples should be performed, with particular focus on chlorinated organics, especially those that might form from the reactions of chlorine with waste products from the poultry. Assessments should then be made of the possible health significance of any identified and quantified organics, including a determination of the likely impact of the quantities to which the consumers of the poultry would be exposed.

3. Although not of primary concern, some effort should be expended on optimizing the renovation system to minimize the unnecessarily high concentrations of some chemicals measured in the renovated water. This includes raising the pH and lowering the sulfate and ammonia levels in the renovated water.

4. In addition to the routine monitoring of turbidity, pH and chlorine already in effect in the renovation system, other parameters should be measured frequently on site to obtain an early indication of unusually high levels of contaminants, at least during the trial period of reuse. Examples are conductivity and total organic carbon.

5. Some consideration and investigation should focus on the possibility of reducing the organic content of the renovated water, either before or after mixing with the well water. However, this must be evaluated, not only in terms of the quality of the water itself, but also the impact on the processed carcasses and the costs as well.

SECTION 4

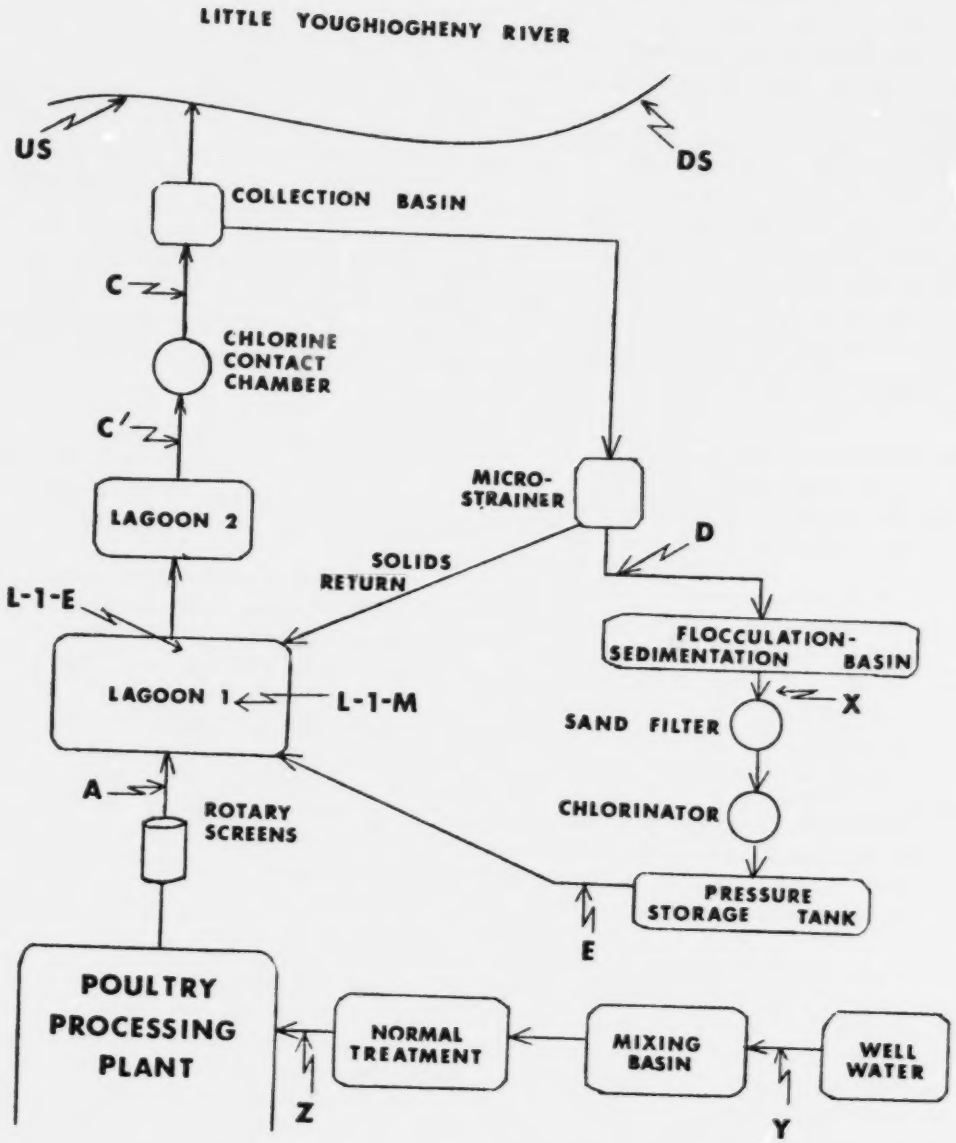
OVERVIEW

Figure 1 is a schematic of the poultry processing plant in relation to its normal source of well water, the aerated lagoons discharging after chlorination to the Little Youghiogheny River, and the diversion of a portion of this discharge to the renovation system. Also shown are various sampling points employed during the study, with their letter reference designations. On occasion other sampling points were utilized, and these are identified on page xi which contains a list of symbols, including those for the chiller waters and the "dressed" poultry, referred to in this report as carcasses.

Poultry, usually chickens, are shipped by truck from the hatchery in Delaware to the Sterling plant and are processed on the day of arrival. Within the plant the birds are slaughtered, scalded, picked, eviscerated, chilled, cut-up and packaged. The poultry processing wastes resulting from the evisceration step are treated by rotary screening for the removal of feathers and viscera. The raw wastewater (sample point A, Figure 1) from the other poultry-processing steps, the refrigeration drains and the plant clean-up passes to a mechanically aerated primary lagoon which is equipped with a grease skimmer. The effluent (sample point L-1-E) from the primary lagoon discharges through a weir trough into a mechanically aerated secondary lagoon (sample point C'), which also contains a grease skimmer. Each lagoon is about 1.8 m deep. The first has a capacity of about 14,000 m^3, and the second about 6,000 m^3. Their combined retention time normally is two to three weeks. The effluent from the secondary lagoon is chlorinated as it passes into a combination settling unit and chlorine contact chamber. The chlorinated effluent (sample point C) is discharged through an overflow weir trough to the Little Youghiogheny River.

As shown in Figure 1, a portion of the chlorinated lagoon effluent is diverted to the renovation system, which in sequence consists of a micro-strainer, flocculation with alum and lime in a basin, followed by filtration through a sand filter and chlorination. During this study the effluent from the renovation system, E, was returned to lagoon 1, as were the solids from the micro-strainer. In actual reuse the renovated water would be mixed with the well water Y, the mixture then to receive the normal treatment currently utilized for the well water alone. This consists of pre-chlorination in the mixing basin, alum-lime flocculation with final pH adjustment to precipitate iron, settling, and filtration through two sand filters. Additional chlorine for residual control is introduced into the main service line leading to the processing plant.

In actual reuse the raw wastewater A, prior to its return to the poultry processing plant, would receive four stages of chlorination: at the effluent



Sample point identification

A, untreated wastewater; L-1-M, lagoon 1; L-1-E, lagoon 1 effluent; C', lagoon 2 effluent, unchlorinated; C, lagoon 2 effluent, chlorinated; US, river, upstream; DS, river, downstream; D, micro-strainer effluent; X, flocculated, settled effluent; E, fully renovated water; Z, normally treated well water.

Figure 1. Schematic diagram of wastewater treatment-renovation system at poultry processing plant with water sampling points.

from lagoon 2; subsequent to sand filtration in the renovation system; and pre-chlorination and post-chlorination in the normal well water treatment system. The renovation system was designed to treat about 50 percent of the flow from the second lagoon effluent. In reuse the renovated water would then be mixed 50/50 (on an equal basis) with the well water, the mixture then receiving its normal treatment prior to use in the poultry processing plant. An important difference between the Phase 2 study and the flow regime in actual reuse is that in Phase 2 the flow through the renovation system was about one-third of that through the processing plant, rather than the one-half planned in actual reuse. Thus in Phase 2, since the renovated water was discharged into the first lagoon, its combined flow with that of the waste-water, A, is one-third higher than the normal waste flow or that expected in actual reuse. This results in a higher dilution of any waste constituent from A emitted to the lagoon system, but at the same time reduces the resid-ence time within it.

The earlier study of Clise (7) indicated that the processing plant was successful in reducing its water usage to about 26 l per bird. For a typical 8-hour shift processing 50,000 birds, this amounts to 1.3×10^6 l of water. Of this total amount about half is used during processing and the remainder for clean-up (7). Probably the most important water exposure of the carcasses is that in the chiller prior to packaging. At the Sterling plant there are two chillers operated in series, with fresh water flowing counter-current to the movement of the carcasses. The typical water temperature in the first is about 13°C, and about 2°C in the second, the total exposure or residence time of a carcass in the combination of the two chillers being about 25 min-utes. Regulations of the U.S. Department of Agriculture limit the water up-take to a maximum of 12 percent of the carcass weight. At the Sterling plant the typical uptake is 6 to 8 percent. Because the chiller constitutes such a critical exposure to water in the plant, a large part of the study focused on the chiller water quality, the possible influence on it of the constit-uents in its finished water source, and the measurement of possible contamin-ants in the carcasses subsequent to their exposures to the chiller system.

The sand filter in the renovation system was constructed to replace the diatomite filter used in previous study (7), and the optimization of the renovation system, Phase 1, began in the Fall of 1975 and continued inter-mittently through the winter as weather conditions permitted. Preliminary sampling for the Phase 2 study began in late February 1976 to optimize the sampling and analytical methodology. This was followed by the full Phase 2 sampling program, approximately once a week for seven weeks from March 15 through May 3, 1976. On each of these days various water and wastewater samples were collected. In addition, twenty-five carcasses were collected for analysis from the plant chiller system. Finally, a small "experimental" chiller was set up to simulate the plant chiller. Twenty-five carcasses were taken from the plant, prior to exposure to the plant chiller, and placed in the experimental chiller in such a way and for such a period as to simulate the plant chiller. However, the experimental chiller was filled with renova-ted water. The purpose was to analyze and compare the possible build-up of contaminants in carcasses exposed to the plant chiller using normal, treated well-water, versus those exposed to the experimental chiller using renovated water. At no time during Phase 2 was renovated water used in the processing plant.

The typical Phase 2 sampling points and the number of samples taken on a typical sampling day are shown in Table 1. Not all of the sampling points were subject to all the analyses. The types of analyses that were performed are shown in Table 2. As shown there, Categories Ia, b, and c were routinely performed only on water samples, while Id was to be done on carcass samples as well. In fact, some Ia and Ib analyses were also performed on carcasses. Category II and III analyses were done on both water and carcass samples. Other sampling points were utilized and analyses not listed in Tables 1 and 2 occasionally performed.

Although analyses of viruses were not contemplated originally in this study, the decision was made to attempt to measure an avian virus and use it as an indicator or sentinel of the behavior of other viruses in the water renovation system. Since it was reported that the chicken flocks were routinely inoculated with live, attenuated Newcastle Disease virus (NDV), it was decided to develop the methodology and sample the water and carcasses for NDV. In addition, some laboratory die-off studies were performed using lagoon water inoculated with NDV.

Finally, and subsequent to Phase 2, total organic carbon measurements (TOC) were performed on the renovated water, the normally treated well water, and that taken at other selected sampling points. In addition, a few samples of the treated well and renovated water were analyzed by gas-chromatograph-mass spectrometry (GC-MS) for some specific organics, other than the pesticides of Category III. Also during one month subsequent to Phase 2, samples were analyzed for trihalomethanes at several points in the water supply system, lagoons, renovation system, and the receiving stream.

TABLE 1. PHASE 2 - SUMMARY OF TYPES OF SAMPLES TAKEN FOR ANALYSES EACH WEEK

Location	Maximum number per day
Water samples	
A - Raw waste	2
E - Renovated water	2
Z - Treated well water	2
PC- Plant chiller	2
EC- Experimental chiller	2
Birds (carcass samples)	
PB- Plant chiller birds	5*
EB- Experimental chiller birds	5*

*50 carcasses were taken for analysis each sampling day, 25 PB and 25 EB. In each case washings from 5 carcasses were composited or otherwise combined to become a single carcass sample. Hence, 25 carcasses reduced to 5 samples.

TABLE 2. TYPES OF ANALYSES PERFORMED ROUTINELY

Category I analyses

Ia - These analyses relate primarily to waste treatment efficiency and none were performed on carcasses routinely

BOD_5	Suspended solids
Grease	Total solids
Organic nitrogen	Alkalinity
Ammonia nitrogen	

Ib and c - These analyses relate to waste treatment efficiency and renovated water quality; none were performed on carcasses routinely

Ib	Ic
Turbidity	CCE (Carbon chloroform extract)
Color	
Total dissolved solids	
Residual chlorine (total)	
Residual chlorine (free)	

Id - These analyses relate to waste treatment efficiency and water quality. They were measured for water and carcass samples.

Total plant count	Fecal coliform
Total coliform	pH

Category II analyses

These analyses were performed on water and carcass samples

Salmonella	Drug residual

Category III analyses

These chemical analyses were performed on water and carcass samples

Arsenic	Copper	Magnesium	Selenium
Barium	Cyanide	Manganese	Silver
Cadmium	Fluoride	Mercury	Sodium
Calcium	Hardness	MBAS	Sulfate
Chloride	Iron	Nitrate	Zinc
Chromium	Lead	Potassium	Pesticides

SECTION 5

METHODOLOGY

Except where noted below all analytical methodology for chemicals and microorganisms is consistent with U.S. Environmental Protection Agency (23, 24) or other standardized techniques (1). All necessary precautions for transporting and preserving the integrity of the samples were taken. This included adding chemical preservatives, storage in the cold, and the use of various types of sample bottles and vials, depending on the nature of the analysis. All water and wastewater samples were individual "grab" type (no compositing was done).

The experimental chiller using renovated (E) water was operated on a batch basis. That is, it was filled with renovated water and plant-made ice was added to bring the temperature initially to about 13°C. The 25 carcasses were then lowered into the bath within the drum and rotation begun. Additional ice to maintain 13°C was added. Approximately 15 minutes later more ice was added to reduce the bath temperature to about 1°C, and chilling continued for another 10 minutes.

The carcasses were removed by handling with clean plastic gloves, and both plant and experimental chiller carcasses were treated in the same fashion. They were placed, after draining, in either clean or pre-sterilized (by autoclaving) plastic bags and carried to the laboratory trailer. 1500 ml of either distilled or distilled and sterile water was added to each such bag, which was then shaken for one minute and the water contents poured for analysis. This rinse sampling method has been used primarily for the detection of bacteria in processed poultry (4, 6), but was employed in this study for both microbiological and chemical analyses of the carcasses.

Trace metals were analyzed by atomic absorption spectrophotometry using solvent extraction to increase the sensitivity. Reference samples to test and improve, where necessary, the accuracy of the analyses were obtained from the EPA and utilized for trace metals, several other inorganic constituents, and pesticides.

A variety of non-standard microbiological methods were utilized and/or modified for Salmonella and Newcastle Disease Virus, as well as the simulated lagoon die-off rate studies for the latter, and these are described in Appendix A, along with the methodology for the residual drug analysis. Similarly, the details of the volatile and non-volatile organic chemical analyses are presented, and the statistical methodology as well.

SECTION 6

RESULTS AND INTERPRETATION

INTRODUCTION

The results presented in this section will focus primarily on Phase 2, at least for the analyses of the inorganic constituents and physical parameters measured in the water, wastewater and carcasses. Additionally a description will be presented of the operation of the renovation system, along with some comparisons with the earlier study of Clise (7) and, where appropriate, use of data from Phase 1 and post Phase 2. Data were also obtained and are presented for many parameters in the chillers during Phase 2. Because of the large amount of data, for the most part the results will be summarized, showing the number of analyses, the means, standard deviations, and in some cases the maximum values. Most of the analyses in Phase 2 were performed on samples taken on each of the seven sampling dates. In some cases, however, and as originally planned, where the results were consistently negative (below the level of sensitivity), the analyses were discontinued.

Statistical analysis of the data is confined mostly to those inorganic constituents and physical parameters for which there were sufficient numbers to be meaningful and useful. In addition to comparing the Phase 2 results with the earlier study of Clise (7), such comparisons have been made as are helpful in determining any differences between the renovated (E), wastewater (A) and treated well water (Z), thereby focusing on possible build-ups in the renovation system or inefficiencies in treatment, as well as possible health concerns. Of special interest also are the statistical analyses and comparisons of carcasses exposed to the waters of the processing plant chiller (PC) and experimental chiller (EC). Their possible differences were determined, and an analysis performed to assess the contributions of constituents in the chiller water to those measured in the carcasses (washings).

Some organic parameters were measured routinely in Phase 2, but these generally were the gross ones (rather than specific organic chemicals) which are reported in tables showing several waste parameters. These include BOD_5 and organic nitrogen. Pesticides were analyzed for a few weeks in Phase 2, but discontinued when the treated well and renovated water samples were all negative. Several CCE results are reported for Phase 2 and other periods. Subsequent to Phase 2 several samples were taken and analyzed at a number of sampling points for TOC and specific organic chemicals, both volatiles (trihalomethanes) as measured quantitatively by GC, and non-volatiles, qualitatively only by GC-MS. In general there were not sufficient organic analyses

for statistical comparisons between Z and E water, although this could be done in one or two cases. However, the organic measurements were of sufficient scope to allow a reasonable assessment of their possible health impact as a result of processing the chickens in the renovated water.

Although coliform, fecal coliform and standard plate count analyses were performed on a variety of water, wastewater and carcass samples, it was decided for the purpose of this report to focus primarily on a comparison of the treated well and renovated waters as a means of evaluating and comparing possible risks. Such bacterial levels in the chillers and carcasses would most likely be unrelated to their respective water sources which are routinely disinfected. In contrast, Salmonella was evaluated in a variety of samples including carcasses, both in Phase 2 qualitatively, and subsequently for its quantitative presence, after the appropriate methodology was developed. Although for all practical purposes there is no basis for concern about avian viruses with respect to human health in this project, Newcastle Disease Virus (NDV) was selected for analysis as a possible sentinel or indicator organism of viral behavior. NDV can cause mild conjunctivitis in humans, but is not known to cause human disease via the gastro-intestinal route. The results of attempts to isolate it in the carcasses and other samples during Phase 2 are described. Additionally, the NDV die-off studies in the laboratory using lagoon water from the Sterling wastewater treatment system are presented and evaluated. Although results were negative, the attempts to assess the presence of antibiotic drugs in various samples using a bacterial drug residual test are described.

Finally there will be presented briefly an analysis of the use of the Phase 2 treatment and renovation scheme as a predictor of the behavior and steady-state build-up of contamination in actual recycle. The detailed calculations and basis for the analysis are described more fully in Appendix B.

OPERATION OF RENOVATION SYSTEM

Phase 1, the optimization of the renovation system, began in the Fall of 1975 and continued intermittently, depending in part on the winter weather conditions, until February 1976 when preliminary sampling for Phase 2 was instituted. Subsequent to Phase 2, starting in May 1976 and continuing to the end of the project in June 1977, additional efforts were directed at improving the operation and performance of the renovation system, such as by pH adjustment and the use of powdered activation carbon.

More detailed information on the lagoon and renovation systems, as well as characterizations of the wastewater, can be found in the report by Clise (7). However, for orientation some of this information is included here. The following material is a summary report of the pertinent aspects of the operation of the renovation system.

Description of Lagoon and Renovation Systems

The wastewater treatment system consists of two lagoons totalling 2.75 acres (1.11 hectares) in area. Each lagoon is 140' (42.7 m) wide. The

primary unit is 590' (177 m) long and the secondary lagoon is 230' (69 m) long. Each pond is six feet (1.8 m) deep. Primary lagoon capacity is approximately 3.75 million gallons (14.195 m^3) and secondary capacity is 1.5 million gallons (5,678 m^3) providing holding capacity for 12 working days' flow.

The primary lagoon is equipped with 64 Link Belt circulators, a grease skimmer, and an effluent wier trough discharging into the second lagoon. Entering at the bottom of the circulators, wastewater is discharged at the surface in one direction, creating a counter-clockwise surface flow. Air is supplied to the circulators by three positive displacement blowers, each powered by a 20 hp (14.9 kw) motor. The system provides 3,360 cfm (15.8 m^3/s) of air at 2.8 psig (19.3 kN/m^2). Air is distributed to the circulators through a header pipe encircling the two lagoons. The secondary lagoon is equipped with 40 Link Belt circulators, a grease skimmer, and a combination settling unit and chlorine contact chamber with an overflow wier trough and discharge line to the river.

Incoming raw wastewater has a BOD_5 averaging 450 mg/1, amounting to a loading approximating 400 lbs/acre/day (448 kg/ha/day) with a 93% reduction in the lagoon system. Raw wastewater suspended solids average 858 mg/1, equaling a loading of 750 lbs/acre/day (841 kg/ha/day) with an 88% reduction in the system.

The advanced water treatment plant was designed to collect the chlorinated effluent from the second lagoon at the point of discharge to the river. Basic design of the advanced water treatment facility consists of a control building; 70 micron microstrainer; a flash mix, flocculation and sedimentation basin; a gravity flow rapid sand filter; gas chlorinator; and 20,000 gallon (76 m^3) pressure storage tank. Supplemental equipment consists of a 3,000 gallon (11,360 liter) concrete pit used as a collection sump for the lagoon effluent; sewage pump for delivery of effluent to the microstrainer; a low head pump used for the application of alum and coagulant aid for delivery of microstrainer effluent to the flash mix unit; high head pump for delivery to the sand filter; chlorine recorder; and electrical control panel. All equipment is automatically controlled by the water height in each unit and is rated at or above 300 gpm (19 liters/sec).

As originally operated, chlorination preceded storage and filtration as a method of assuring breakpoint chlorination. In the later phases of the project, piping changes were made to allow all physical treatment to occur prior to chlorination. These changes were for the purpose of reducing as far as possible the organic content of reclaimed water available for combining with chlorine. Concurrent with this change, powdered activated carbon was introduced in the flash mix for the removal of combined chlorine in the sedimentation process.

Operating Procedures

During this study there were two pumping regimes, continuous and intermittent. The initial pumping operation was to determine capability to meet the demand under the adjusted pumping rate necessitated by balancing four pumps in continous operation. Previous operations required only two units

based on the original design. With the new adjustment the low head pump established a rate for normal operation at 210 gpm (800 liters per min), compared to the designed rate of 300 gpm (1140 liters per min). The system remained under automatic control from pressure in the storage tank with continuous discharge into the No. 1 lagoon.

During Phase 2 the expected demand of 180 gpm (680 liter per min) by the Sterling plant in actual reuse was simulated by discharging this volume of filtered water from the storage tank to waste into the No. 1 lagoon. Normal water requirement in the Sterling plant for a 16 hour day is 288,000 gallons (1,130,000 liters). The new pumping rate in the water re-use plant will give 202,000 gallons (765,000 liters). Under this program the high lift pump was in intermittent service as pressure varied in the storage tank between 55 and 70 psi. The pump cycle was 15 minutes in service and 12 minutes off with continuous flow through the sand filter. All raw water pumps in the Sterling system are also on automatic control. The No. 3 pump at 175 gallons per minute (656 liters per min) will be utilized with the re-use pump at 180 gpm (675 liters per min). The re-use line is metered and proper adjustment can be maintained. The other two Sterling pumps totaling 190 gpm (713 liters per min) will remain in standby service. The re-use line will discharge into the Sterling raw water sedimentation basin through a float valve thereby maintaining a more or less controlled equal volume from the two systems. The re-use water plant would be in continuous automatic service.

The sedimentation basin was drained and washed on a cycle of 2 million gallons (7.6 million liters). This was usually done every other weekend when the poultry processing plant was not operating. During the initial part of the study the filter averaged 5.1 hours of service between backwashings at 8.4 feet (2.6 meters) of head loss. When simulating the reuse operation it averaged 7.0 hours of service at 9.1 feet (3.4 meters) of head loss.

Chemical Treatment and Monitoring

In June 1976 (subsequent to Phase 2) an automatic turbidimeter was placed in service to monitor the renovated water (E). During a period of 93 days the hourly mean value was 1.08 Jackson turbidity units, and the maximum was 2.7.

Chlorine was added typically at a rate of 6.5 pounds (2950 grams) of chlorine per hour, approximately 0.35 pounds (160 grams) per 1000 gallons or 44 mg/l from January 6 to June 3, 1976, which included the Phase 2 period. "Break point" chlorination was accomplished; the free residual chlorine in the microstrainer effluent (243 tests) averaged 0.36 mg/l, with a total residual of 0.84 mg/l, indicating that 0.48 mg/l was present as combined chlorine, probably chloramines. With subsequent use of carbon, the residual chlorine was 0.2 mg/l (243 tests). The post-chlorination dose averaged 1.7 mg/l, with a free chlorine residual of 0.22 mg/l in the renovated water.

The automatic chlorine recorder was placed in service in June 1976. Operating on post-chlorination, with 305 tests reported, the average free residual in the filter effluent was 1.9 mg/l, with a minimum of 0.5. Diethyl-p-phenylene diamine (D.P.D.) replaced orthotolidine arsenite (O.T.A.) as the procedure for residual chlorine on March 29, 1976. The automatic

chlorine recorder gave results in agreement with the D.P.D. test.

To eliminate chlorine and chloramine fumes in the control house, activated carbon at 10 mg/l was applied to the flash mixing tank beginning June 21, 1976. This was highly effective, as shown by the chlorine residual measurements, which indicated that one-third of the measured values were between 0.1 and 0.2 mg/l, and the remainder were below the sensitivity limit.

Under normal conditions the suspended solids in the lagoon system effluent varied from 12 to 88 mg/l with a mean of 40 mg/l. Coagulant dosage was determined by daily or more frequent "jar tests." With normal raw lagoon water it averaged 15.4 mg/l alum, 5.3 mg/l lime, 1 mg/l polymer and 10 mg/l carbon. Due to seasonal turnover of the lagoons from October 19 to December 13, 1976, the suspended solids of their effluent averaged 304 mg/l with a minimum of 114 mg/l.

A solid ice cap on the lagoons between December 13, 1976 and March 8, 1977 prevented operation of the plant. From April 25 to June 8, 1977 a second seasonal turnover occurred. In 1976 the dissolved oxygen was depleted but the lagoons did not become anaerobic. However, in 1977 the lagoon became anaerobic with resultant development of putrescible sludge and hydrogen sulfide killing the organisms necessary to stabilize the deposited sludge.

During the period while the plant was out of service a series of jar tests (147) was made to develop a coagulation procedure that would produce compact, settleable floc. They indicated that a dosage of 22.2 mg/l alum per percent of settleable solids at a pH value of 6.3 would be practical. However, during this operation screens would have to be removed from the microstrainer and excessive backwashing of the sand filter on a 4 hour cycle would be required. At normal pumpage this dosage is equivalent to 821 mg/l of alum, 1382 mg/l lime, 1 mg/l of polymer and 10 mg/l of carbon. These dosages greatly exceeded the capacity of the 308 mg/l feeding equipment, which was designed for 7.8 mg/l hexane soluble material, 58 mg/l suspended solids and 30 JTU turbidity. It was not possible to operate the plant during this period. Supplementary chemicals such as hydrogen peroxide and excess chlorine were also employed without appreciable effect. Based on this effort and analysis, it was judged that operation of the reuse system should be suspended during a period of lagoon turnover.

Hexane soluble material was found in the renovated water effluent at concentrations varying from 0.2 to 6.0 mg/l. The average of 28 tests was 3.6 mg/l. Recent concentrations of hexane soluble material found in the three Sterling wells ranged from 4.0 to 4.8 mg/l. These wells are in the Pocono formation of the Deer Park anticline, which is generally sandstone. They vary from 168 feet (53 meters) to 362 feet (112 meters) in depth. The hexane soluble content of three other wells, ranging in distance from 15 meters to 5.5 kilometers from the Sterling plant, varied from 0.4 to 2.6 mg/l. There are abundant gas wells and coal seams in the vicinity at depths which by lateral diffusion could readily influence the organic content of the Sterling and other nearby wells.

PHYSICAL AND INORGANIC CONSTITUENTS

Finished Water and Wastewater

The summary of the Phase 2 results for several physical and macro inorganic constituents of Z, A and E waters is presented in Table 3, along with the EPA Interim Primary and proposed Secondary Drinking Water Regulation constituent limits (25, 26). In that table the former include only turbidity and nitrate, the others being Secondary regulation limits. The nitrate maximum constituent limit was not exceeded in the E water in Phase 2, and the mean of 3.5 mg/l was considerably below that limit of 44 mg/l (as NO_3^-). Subsequent to Phase 2 an additional 24 samples of E water had a mean concentration of 2.16 mg/l, lower than that in Phase 2.

The current EPA turbidity limit is one, rather than the five shown in Table 3. However, five is permitted in certain instances, such as when it does not interfere with disinfection or microbiological determinations. In addition to the turbidity results for E in Phase 2 with a mean value of 1.6, 140 such analyses in Phase 1 had a mean of 2.9 units, and 175 analyses post Phase 2, 2.2 units. It is apparent that, although generally higher than the limit of one, they are less than five; also, as will be discussed subsequently, the disinfection processes were quite effective.

The pH and alkalinity in E, the renovated water, during Phase 2 were significantly lower than that of Z, the treated well water, which for these parameters was quite similar to A, the raw wastewater. These low values in E can be attributed reasonably, at least in large part, to the extensive chlorination, both of the secondary lagoon effluent and the renovation system, as well as the addition of alum as a coagulant. The chlorine applied typically to the former was 25 mg/l (7), and 44 mg/l to the latter during Phase 2. The application of this total amount of chlorine generates enough acid to neutralize about 50 mg/l alkalinity (calcium carbonate) and, at the same time lowers the pH. In the previous study (7), as shown in Table 10, the average pH was higher, namely 6.6, compared to 5.8 in Phase 2. Also, in about a one year period subsequent to Phase 2 the average of 167 pH measurements was 6.06, with a standard deviation of 1.02. Although there is no direct health risk from such relatively low values of pH, to the extent that this could adversely affect treatment or cause corrosion, it should and could be rectified relatively easily by the addition of soda ash. In actual reuse there will be mitigation of this potential problem, in any event, due to the 50/50 mixing with well water.

As shown in Table 3, the average free chlorine residual during Phase 2 was 1.7 mg/l in the renovated water. Subsequent to this period over 300 readings showed an average of 1.9 mg/l, and a minimum of 0.5. Such chlorine levels, maintained consistently in conjunction with its application elsewhere in the system, would be expected to provide a high degree of assurance against risks from pathogenic microorganisms in actual reuse.

Of the remaining parameters the only one occasionally approaching a recommended (esthetic or secondary) constituent limit in the renovated water is sulfate. The mean concentration of 150 mg/l is considerably below

TABLE 3. PHASE 2 - WATER QUALITY CHARACTERISTICS AND MACRO CONSTITUENT CONCENTRATIONS[±] IN Z, A AND E
(in mg/l, except where noted)

	Treated well water (Z)				Screened raw wastewater (A)				Renovated water (E)				EPA interim criterion
	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max	
Turbidity (JTU)	6	0.9	0.5	1.7	-	-	-	-	35	1.2	0.65	2.8	5
Color (units)	6	3.0	1.6	5	-	-	-	-	36	3.7	3.3	10	15
*pH	12	7.0	0.2	7.2	10	7.1	0.2	7.3	30	5.8	1.4	6.9	-
*Alkalinity	12	107	15	130	10	113	50	174	15	35.3	37	140	-
Chlorine res.	-	-	-	-	-	-	-	-	52	1.7	1.3	6.0	-
Surfactants (MBAS)	14	<0.01	-	-	15	0.27	0.18	0.71	13	0.04	0.02	0.08	0.5
*Na^+	14	7.4	3.4	12.5	16	62.4	56.4	203	14	30.2	12.5	47.7	-
*K^+	16	2.9	3.6	8.7	15	32.2	10.0	47.4	15	14.7	2.3	17.4	-
*Ca^{++}	16	40.1	4.8	49.4	16	42.8	7.4	50.8	16	53.7	5.8	64.9	-
*Mg^{++}	16	2.4	0.3	2.9	12	4.0	0.8	5.2	15	3.0	0.3	3.4	-
*Cl^-	12	11.6	1.0	13.4	14	62.2	24.2	91.1	12	88.5	16.8	121	250
NO_3^-	8	4.0	0.6	4.9	8	0.15	0.041	0.18	8	3.5	1.5	6.2	44
*SO_4^{--}	8	9.6	3.6	13.5	8	15.7	4.7	33.0	8	150	85.6	252	250
HCO_3^- (est)	-	130	-	-					-	43	-	-	-
Ion total		208								393			
*Dissolved solids	12	143	33.8	194	10	748	410	1850	16	389	66	492	500

[±]Occasionally extreme values (beyond 2 S.D.) were discarded.

*The mean values for these parameters were statistically different in Z and E at alpha 0.05.

the recommended limit of 250 mg/l, although one value at 252 mg/l exceeded it marginally. Although not a health concern, the build-up could be reduced by modification of the use of alum as a coagulant, a major contributor to the sulfate in the renovation process.

The 389 mg/l average for the total dissolved solids in the renovated water during Phase 2, shown in Table 3, was considerably higher than that of the treated well water, although less than the non-mandatory criterion value of 500 mg/l. Also, as shown in Table 3, the sum of the mean values of the concentrations of the cation and anion constituents is essentially identical with that for the dissolved solids. One can thus conclude that it is unlikely that any unknown or unidentified constituents are making substantial contributions to the dissolved solids content of the renovated water. Nevertheless, it is apparent that there are large concentration increases for several of these inorganic ion constituents in the renovated water compared to the treated well water, even though at the levels encountered they do not pose a health hazard. The listing in Table 3 of the various parameter statistics for the wastewater, A, provides a perspective as to the possible sources of constituents that are measured in E. Thus, for example, since the average nitrate concentration of 3.5 mg/l is substantially higher than the 0.15 mg/l level in the raw wastewater, one can reasonably conclude that this is due to biological nitrification in the lagoons of the various other nitrogen sources in the waste effluent shown in Table 4.

The Phase 2 summary statistics in Z, A, and E for the parameters related primarily to waste treatment are shown in Table 4. With the exception of ammonia nitrogen, there is a considerable reduction in concentration in each case in the E water compared to the raw wastewater, A. The relatively high ammonia nitrogen concentration of 19 mg/l (average) in E is of some interest and possible concern. It probably results from biological decomposition of organic nitrogen constituents in the lagoons, although some concentrations measured in the raw waste are also high. The principal concern is that the ammonia reacts with the chlorine disinfectant to form chloramines, which are less effective disinfecting agents. However, because of this very reaction, unless the samples are analyzed immediately, it is unlikely that with the practice of breakpoint chlorination the ammonia should have been detected. Subsequent to Phase 2 the ammonia in E decreased considerably, the average concentration for 10 samples being 5.1 mg/l. This could have been a result of an increase in nitrification in the lagoons during periods of warmer temperature. It should be emphasized that the low bacterial counts in the E water indicates that disinfection has not been affected. Also the presence of these concentrations of ammonia is not known to be a health hazard. Finally, it is noteworthy that ammonia is sometimes added in municipal water treatment plants in order to react with chlorine and form longer-lived chloramines.

The organic nitrogen concentrations in the renovated water are substantially higher than in Z in Phase 2, although the average 3.4 mg/l BOD in E does not reflect this difference. For such measurements taken over an 18 month period, including and subsequent to Phase 2, the overall mean BOD for E was 4.2 mg/l, still less than the 5.3 mean value for Z shown in Table 4.

TABLE 4. Z, A AND E WATER CHARACTERISTICS RELATED PRIMARILY TO WASTE TREATMENT EFFICIENCY (in mg/l)

	Treated well water (Z)				Screened raw wastewater (A)				Renovated water (E)			
	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max
*Total solids	12	165	33	234	10	1380	324	2074	13	418	72	501
Suspended solids	12	10.4	8.1	26	10	543	131	690	14	16.8	19.7	70
BOD_5	12	5.3	4.3	18	10	377	184	649	14	3.4	2.2	7.0
Grease	12	5.2	6.1	23	10	164	44	260	13	5.1	4.8	18.2
*Organic-N	8	0.013	0.008	0.030	5	46.3	26.5	81.3	10	1.7	1.5	5.0
*Ammonia-N	8	0.017	0.013	0.040	5	6.2	1.2	7.9	10	19.0	4.4	23.0
*Dissolved oxygen	12	8.1	0.6	9.4	10	8.0	0.54	8.8	14	9.3	1.4	11.7

*The mean values for these parameters were statistically different in Z and E at alpha 0.05.

A summary of the results for the trace constituents in Z and E waters during Phase 2 is shown in Table 5. It should be emphasized that the concentration units here are micrograms per liter. All of the maximum concentrations were well below the criterion values, except for lead in both E and Z samples. They were not greatly different for the two waters, and the high concentrations in each case were just about at the criterion limit. Only in the case of iron and fluoride were the concentrations substantially higher for E compared to Z. However, it is judged that for none of these six trace elements were there any hazards in the renovated water, since, except for the one high value for lead, they were well below the criterion limits.

As indicated in Table 5, all the measurements for silver, arsenic, cadmium, chromium, and selenium were negative (below the sensitivity limits). About half of the E samples were positive for cyanide at concentrations up to 12 µg/l, but well below the health criterion value of 200 µg/l. It is difficult to imagine any oxidizable cyanide being present in E because of the large quantities of added chlorine. However, the analysis was for total cyanide, so that the measurement may have detected such harmless combined complexes as those involving iron, often used as an anti-caking agent, such as in road salt. Several water samples were positive for mercury. These occurred in three of the eight weeks, two of which were analyzed on the same day. It is likely that in the latter cases there was contamination or analytical error, perhaps as a result of the mercury preservative added to the nitrate sampling bottle. On two subsequent weeks when this possibility was removed, only one out of six E samples were positive (0.6 µg/l), and no Z samples. In contrast, during the previous two weeks E, Z and all other samples were positive and much higher. For the samples of the first four weeks none of the E or Z samples were at concentrations higher than 0.2 µg/l, the level of sensitivity of the method. In view of the fact that the highest E sample was 1.7 µg/l, even though it was probably an erroneous reading due to contamination, and the fact that the criterion value is 2 µg/l, it may be concluded that there is not observed health hazard from mercury in the renovated water.

Chiller Water

Although a principal focus of this study is the possible difference in the water quality of the renovated and treated well waters, it was recognized that it would be useful also to analyze the plant and experimental chiller waters during Phase 2. The results of these analyses for several macro and trace constituents are presented in Tables 6 and 7, respectively. Although the experimental chiller was operated, as described in Section 5, in such a fashion as to simulate the carcass exposure to such water in the actual plant operation, there was one important difference. In the poultry plant the chiller was operated continuously and counter-currently, while the experimental chiller was on a batch basis. Thus in the plant carcasses continuously moved through the chiller at the same time that fresh water was continuously added. In the experimental chiller a single group of 25 carcasses was added to a given volume of water. Thus the exposure of the carcasses in these two situations and their uptake of chemicals or other constituents would likely be somewhat different; similarly, the relative quantities of such materials leaching from the carcasses into the chiller water would also be expected to differ.

TABLE 5. PHASE 2 - TRACE CHEMICAL CONCENTRATIONS± (ug/1) IN Z, A AND E

	Treated well water (Z)				Screened raw wastewater (A)				Renovated water (E)				EPA interim criterion limits
	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max	
Cu	16	41.4	11.2	57	14	49.0	31.1	147	14	38.8	12.9	56	1000
*F	14	58	33	125	12	128	42	207	14	151	54	230	1400 - 2400
*Fe	16	19.2	7.8	31	16	44.3	15.1	91	14	57.1	24.3	98	300
*Mn	16	1.8	1.0	3	12	5.8	3.4	16	14	2.6	1.0	4	50
Pb	16	21.2	16.7	50	16	48.7	31.9	141	15	23.8	13.9	50	50
Zn	12	25.2	3.8	33	16	41.9	29.3	108	12	27.2	6.9	38	5000

±Occasionally extreme values (beyond 2 S.D.) were discarded.

*The mean values for these parameters were statistically different in Z and E at alpha 0.05.

Note

The following elements were in all cases below the indicated sensitivity limits:

Ag - 10-20 ug/l
As - 10 ug/l
Cd - 10 ug/l
Cr - 30 ug/l
Se - 10-350 ug/l

The following elements were unusual in certain respects:

Ba - analyses were performed at the sensitivity limit; results unreliable

CN^- - Z samples were negative; 9 of 16 E samples were positive at concentrations ranging from 1 to 12 ug/l; 2 of 15 A samples were positive, each at a concentration of 2 ug/l.

Hg - several samples were positive for mercury at concentrations up to 3 ug/l, but the positive results may be due to contamination.

TABLE 6. PHASE 2 ANALYSES OF CONCENTRATIONS (mg/1) OF MACRO CONSTITUENTS IN WATER FROM PLANT (PC) AND EXPERIMENTAL CHILLERS (EC)

Parameter	Plant chiller (PC)			Exptl. chiller (EC)		
	N	$\bar{X}$	S.D	N	$\bar{X}$	S.D
Surfactants	12	0.22	0.14	7	0.047	0.023
Calcium	12	39	9.3	9	58	7.8
Magnesium	12	3.4	4.9	9	3.0	0.22
Potassium	12	37	11	9	25	6.9
Chloride	11	120	32	9	197	89
Sodium	12	80	26	9	95	43
Nitrate	6	0.89	0.96	5	2.7	1.0
Sulfate	6	14	6.8	5	167	53

Note

With the exception of sodium, the means for each parameter in PC and EC are statistically different at alpha 0.05.

There are two important comparisons to make in evaluating and interpreting the chiller analyses. The first is that between the plant (PC) and experimental chillers (EC), and the second between the chillers and their corresponding water sources, Z and E. In the first case for the macro constituents reported in Table 6 there are some obvious large differences in mean concentrations, with, for example, calcium, chloride, potassium and sulfate being much larger in EC than PC. At the same time a comparison of these same parameters in their respective water sources, E and Z, shown in Table 3, indicates that the calcium and sulfate concentrations are not very different (EC vs. E, and PC vs. Z); in contrast, for chloride and potassium the chiller waters are much higher. It is not unreasonable to conclude that for these latter ions the carcasses themselves are probably contributing to the chiller waters by a leaching or similar mechanism. This is confirmed by the concentrations for these ions in the wastewater A, shown in Table 3, where

TABLE 7. PHASE 2 ANALYSES OF CONCENTRATIONS (µg/1) OF TRACE CONSTITUENTS[a] IN WATER FROM PLANT (PC) AND EXPERIMENTAL CHILLERS (EC)

	Plant chiller (PC)			Exptl. chiller (EC)		
Parameter	N	$\bar{X}$	S.D	N	$\bar{X}$	S.D
Copper	11	47	20	8	30	11
Iron	12	48	14	9	40	24
Lead	12	55	41	8	21	7
Fluoride	8	88	37	9	130	59
Zinc	11	29	10	9	32	24
Cyanide	See note b			7	4.3	2.7
Selenium	5	200	56	See note c		

Statistical comparisons of the means of all parameters in PC and EC show no differences at alpha 0.05

[a] In each case the analyses for the following trace elements were below their respective sensitivity limits shown in parantheses in mg/1. Arsenic (0.01); cadmium (0.01); chromium (0.03); silver (0.02). Mercury: for PC, 2 of 10 samples were positive with concentrations of 0.4 and 1.5 µg/1; for EC, 2 of 16 were positive with concentrations of 0.2 and 0.5 µg/1.

[b] For cyanide in PC, 2 of 10 samples were positive with concentrations of 1 and 2 µg/1.

[c] For selenium in EC, 1 of 3 samples was positive with a concentration of 170 µg/1.

they are substantially higher than those of the well water Z. These types of interrelationships will be examined in more detail later in this section.

The trace constituents in the two chillers showed variable behavior, as can be seen in Table 7. When comparing PC and EC, the mean concentrations were not substantially different for iron and zinc. Copper and lead were higher in PC, and fluoride higher in EC. Iron, fluoride and lead are much higher in PC versus Z. Since the wastewater A is also much higher for these elements than is Z, it is clear that the carcasses are contributing substantially to the chiller concentrations. However, of the three, this only seems to apply to lead when making similar comparisons between EC and E.

Carcasses

Probably the most important results are those that assess any possible differences between the quantities of constituents measurable in the washings from chickens exposed to the normally used well water in the plant versus those similarly exposed to the renovated water in the experimental chiller. These results are presented in Tables 8 and 9 for the former (PB) and the latter (EB) for the macro constituents, waste type parameters and trace elements. Most of the physical characteristics and inorganic constituents measured for the Z and E water and shown in Tables 3, 4 and 5 were also analyzed in the carcasses. The macro constituents shown in Table 8 were very similar for PB and EB in every instance. The same applies to the waste type characteristics of Table 9, with the possible exception of ammonia nitrogen, which was somewhat higher in EB than PB. This could very well have been influenced by the high ammonia concentrations in the renovated water, and will be considered in more detail later in this section. Although five trace constituent analyses are reported in Table 9, analyses were also performed for silver, arsenic, cadmium, chromium, and selenium. However, for each of the latter the results were below the limit of sensitivity. For the five shown in Table 9 the mean values for PB and EB were quite similar, with the possible exception of zinc, which was lower in EB. One can at this point make the preliminary judgment that, for almost all the measured physical and waste type parameters, as well as macro and trace inorganic constituents, there are no important differences between the carcasses processed in the plant and experimental chillers. This is, however, considered more fully subsequently, where the statistical comparisons are discussed, as well as the more detailed possible interrelationships of finished water, chiller water and carcass constituents.

STATISTICAL AND OTHER COMPARISONS

Using the two-tail t-test described in Appendix A, comparisons were made of the statistical differences of the means of many of the physical characteristics, waste parameters and trace and macro inorganic constituents in Z versus E, PC versus EC, and PB versus EB. Additionally these were also compared, where appropriate, for this same set of parameters in the renovated water during Phase 2 versus that in the earlier study of Clise (7), and these are shown in Table 10. Of the parameters listed there, the only means that were statistically identical were those for total dissolved solids, potassium, sodium and fluoride. Of the ten parameters whose means were

TABLE 8. PHASE 2 - COMPARISON OF CARCASS ANALYSES (WASHINGS) FROM PLANT AND EXPERIMENTAL CHILLERS - MACRO CONSTITUENTS (mg/l)

	Plant birds (PB)				Experimental birds (EB)			
	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max
Surfactants (MBAS)	21	0.081	0.075	0.27	16	0.052	0.032	0.12
Dissolved solids	6	220.3	51.1	320	6	222.5	68.7	320
Ca^{++}	34	5.3	2.3	12.0	34	5.1	2.1	9.9
K^{+}	34	17.9	8.4	40.6	34	15.6	3.9	28.0
Na^{+}	34	27.4	20.1	78.6	34	24.1	16.9	61.5
Mg^{++}	29	0.57	0.37	1.5	26	0.52	0.44	2.3
Cl^{-}	34	26.5	9.1	46.4	34	24.7	4.1	32.5
NO_3^{-}	6	0.20	0.13	0.44	6	0.17	0.075	0.31
SO_4^{--}	8	9.53	4.63	15.0	12	8.34	5.43	18.6

Note

Statistical comparisons of the means of all the above parameters in PB and EB show no differences at alpha 0.05.

different, four were higher in Phase 2, namely calcium, sulfate, iron and lead. There were a few differences in the nature and operation of the renovation system in the two studies. However, in neither study could any of these parameters at the levels encountered be regarded as risks to health.

The results of the two-tail t-test comparions for Z versus E only in Phase 2 are noted in Tables 3, 4 and 5. In Table 3, for those parameters which could be compared, only the means for turbidity, color and nitrate were not statistically different. For those which were distinguishable, only pH and alkalinity were lower in E compared to Z. The likely reasons for these lower values in terms of chlorine addition were discussed earlier, as were the sources of the other increments that would yield higher levels of constituents in E. Of the seven waste characteristic type of parameters in Table 4, suspended solids, BOD and grease were statistically indistinguishable for Z and E. The dissolved oxygen differences are not of great interest, and the possible reasons for the others have been discussed. Of the six trace elements shown in Table 5, fluoride, iron and manganese could be distinguished statistically, and in each case was higher in E than in Z. For these three the higher mean concentrations in A compared to Z indicate that the raw waste itself is contributing greatly to the increased levels in E.

TABLE 9. PHASE 2 - COMPARISON OF CARCASS ANALYSES (WASHINGS) FROM PLANT AND EXPERIMENTAL CHILLERS

	Plant birds (PB)				Experimental birds (EB)			
	N	$\bar{X}$	S.D.	Max	N	$\bar{X}$	S.D.	Max
	Waste characteristics (mg/l)							
Total solids	6	269	53	372	6	245	68	338
Suspended solids	6	57	25	80	6	35	18	56
BOD_5	6	82	17	101	6	67	33	109
Grease	6	41	21	60	6	25	12	40
Organic-N	3	5.7	1.4	6.7	2	8.1	2.7	10
Ammonia-N	3	0.43	0.093	0.49	2	1.1	0.1	1.2
Alkalinity	6	27	9.0	44	6	21	6.7	32
	Trace elements (ug/l)							
Copper	32	15	10	53	32	18	14	69
Iron	33	22	14	50	34	30	27	112
Manganese	30	2.7	1.3	6	30	4.3	1.6	8
Lead	34	28	26	90	35	30	27	96
Zinc	27	27	26	105	29	16	4.8	31

Note

Statistical comparisons performed only for the trace elements show no differences for the means in PB and EB with the exception of zinc at alpha 0.05.

TABLE 10. A COMPARISON OF PHYSICO-CHEMICAL CONSTITUENTS OF RENOVATED WATER (E) IN THE PREVIOUS STUDY AND PHASE 2 (mg/l)

Constituent	Previous study (7)			Phase 2		
	N	$\bar{X}$	S.D	N	$\bar{X}$	S.D
Turbidity*	54	3.5	1	35	1.6	1.7
Color*	15	90	5	36	3.7	3.3
pH*	207	6.6	1.4	30	5.8	1.4
Dissolved solids	158	335	129	16	389	66
Alkalinity*	101	104	57	15	35	37
Potassium	20	11	9.5	15	15	2.3
Sodium	19	21	15	14	30	13
Calcium*	26	46	12	16	54	5.8
Chloride*	162	117	53	12	89	17
Nitrate*	89	31	8	8	3.5	1.5
Sulfate*	23	13	5	8	150	86
Fluoride	23	0.21	0.13	14	0.15	0.054
Copper*	26	0.06	0.01	14	0.038	0.01
Iron*	27	0.27	0.19	14	0.057	0.024
Lead*	8	0.01	0.01	15	0.024	0.014
Manganese*	10	0.02	0.02	14	0.0026	0.001

*The mean concentrations for these constituents were statistically different at alpha 0.05; all others were indistinguishable.

Note

In both studies in almost all cases the concentrations for the following constituents were below the sensitivity limit: cadmium, chromium, mercury, selenium, silver, and pesticides. In Phase 2 only there were a few positive concentrations of cyanide up to 12 µg/l.

The results of the one-tail t-test comparing Z, A and E for all the relevant parameters listed in Tables 3, 4, and 5 are shown in Table 11. It must be emphasized that this statistical test is a measure of which parameters are larger, not merely which are different. The first four relationship categories listed in Table 11 correspond to waters with E parameters larger than those for Z. However, among these categories the A concentrations bear a different relationship to Z and E, reflecting, therefore, different likely causes for the higher concentrations in E. Thus, for the first two ($A > E > Z$ and $A = E > Z$) where A is greater than or equal to Z, the contributions from the raw waste are the probable source. For the third ($E > Z = A$) other factors, involving incremental sources subsequent to the raw waste emission, are responsible. For calcium, this can be attributed to lime addition in the renovation system. For the fourth category ($E > A > Z$) it is also apparent that there are incremental sources between A and Z. These likely sources have been discussed previously for ammonia, chloride and sulfate. Corrosion could account for the increase in iron.

The next four relationships, with the means for E equal to those for Z, also each involve a different relationship to A. For the first ($A > E = Z$) there is an incremental load in the waste which is reduced subsequently, either in the lagoons or renovation system. This could also and probably does apply to the next relationship ($E = Z$) for color and turbidity, but data for A were not available. Copper was the only parameter indistinguishable in all three waters ($A = E = Z$), while nitrate was lower in A, but equal in E and Z ($A < E = Z$). As discussed earlier, nitrification-denitrification processes were probably in effect in the lagoon systems, and could account for these nitrate relationships, along with decomposition of the proteinaceous matter in the wastewater. The likely influences on pH ($E < Z = A$) have been discussed.

As noted in Tables 6 and 7 for the chiller waters, PC and EC, the two-tail t-test indicates that for the macro constituents the means were all different, with the exception of sodium; in contrast, the five trace element means were indistinguishable. Finally, and also as noted in Tables 8 and 9, similar comparisons of the processed carcasses show no statistical differences between the mean concentrations of any of the macro or trace constituents, with the exception of zinc, which was higher in the carcasses (PB) processed in the plant chillers. Statistical comparisons were not similarly made for the waste type parameters listed in Table 9, although ammonia is higher in EC, and this has been discussed.

For the most part there are no significant differences, with the exceptions noted, between the means of the various physical and inorganic chemical parameters for the carcasses, PB and EB, processed in the plant and experimental chillers, in spite of such differences for the comparable constituents in their respective water sources, Z and E, and chillers, PC and EC. The question arises then as to why the latter exert very little, if any influence on the constituents measured in the carcasses. It is possible that for some such parameters any uptake by the carcasses is irreversible in that there is sorption onto or into the flesh, without subsequent leaching in the relatively short period in which the carcasses are analyzed by equilibration (washing) with distilled water. To the extent that this may occur, this

TABLE 11. RELATIONSHIPS BETWEEN CONCENTRATIONS OF PARAMETERS IN PHASE 2 AT THREE SAMPLING POINTS (E, Z, AND A) AS OBTAINED FROM THE ONE-TAILED STATISTICAL TESTS (AT ALPHA 0.05)

Relationship	Parameter
A >E >Z	Dissolved solids, Magnesium, Manganese, Organic-N, Potassium, Sodium, Total solids
A = E >Z	Fluoride
E >Z = A	Calcium, Dissolved oxygen
E >A >Z	Ammonia-N, Chloride, Iron, Sulfate
A >E = Z	BOD_5, Grease, Lead, Suspended solids Zinc
E = Z	*Color, *Turbidity
A = E = Z	Copper
A< E = Z	Nitrate
E< Z = A	pH

*No data for raw wastewater, A, collected for this parameter

Note

A = Raw waste
E = Renovated water
Z = Treated well water

could not be detected directly by the measurements performed in this study. However, as shown in Table 11, only rarely, such as for nitrate, was the concentration of a parameter in the raw wastewater, A, lower than that in Z, the treated water source for the poultry processing plant. Thus, it is reasonable to infer in most instances that instead of a net sorption there is likely to be a net leaching from the carcasses to the chillers, and then to the wastewater.

A more direct way to assess this question is through consideration of the possible mass-balance relationships for the parameters analyzed in the carcass washings relative to the chiller and related finished water sources. By regulation of the Department of Agriculture the maximum allowable uptake of water by the carcasses in the chiller is 12 percent. At the Sterling plant a more typical uptake is 6 to 8 percent. The sampling procedure involved shaking each carcass, after its exposure to the chiller, with 1500 ml of distilled water which presumably mixed or equilibrated with water taken up by the carcass from the chiller. After the mixing, the washings were collected for analysis. During Phase 2 the average carcass ("dressed") weight was approximately 1.3 kg (about 2.9 lbs). Assuming a maximum uptake of 12 percent water in chiller, this corresponds to 156 ml (0.12 x 1300). It can then be assumed that this 156 ml mixes completely with the 1500 ml of the added distilled water, so that to the extent that any constituents are present in the water taken up by the carcasses from the chillers, they become diluted in the washings by a factor of 156/(1500 + 156) which equals 0.0942. If there is simply a dilution of the chiller water taken up directly by the carcasses, and no other factors are operating, then the carcass washings PB and EB, should equal, for any parameter, the concentration in their respective chiller, PC and EC, multiplied by 0.0942. Less likely, but at least worthy of examination, is a similar possible relationship between the finished waters and the respective carcass washings.

The results of such calculations based on this assumption of simple dilution are shown in Tables 12 and 13. In the former, for several physical characteristics, waste type parameters, and one metal, manganese, this was done only for Z and E, not PC and EC, since the latter measurements were not available. These calculated (theoretical) concentrations are shown in Table 12 for the processing plant and experimental chiller. With the exception of ammonia in the experimental chiller, it is clear that in each case the quantities measured in the carcasses (washings) were considerably greater than those calculated from exposures to the respective water supplies for the corresponding chillers. The ammonia in the carcasses in the experimental chiller could indeed have been influenced by that in E water. Also, if instead of the maximum allowable 12 percent water uptake, the more realistic 6 to 8 percent were used in the calculation, this would correspond to an even larger dilution factor and lowering of the calculated value of 1.8 mg/l for ammonia to 0.9 to 1.2 mg/l, very close to that actually measured on the average in EB during Phase 2. In addition, the fact that for none of the parameters listed in Table 12, including ammonia, were the mean concentrations for the carcasses significantly different, in spite of such differences for five of the eight parameters in the Z and E waters, lends further support to the conclusion that the latter waters were not making significant contributions to the levels in the carcasses.

TABLE 12. COMPARISONS OF SEVERAL PHASE 2 ANALYSES OF CARCASSES (WASHING) IN PLANT AND EXPERIMENTAL CHILLERS WITH THEORETICAL CONCENTRATIONS (mg/1) CALCULATED FROM THEIR RESPECTIVE WATER SOURCES, Z AND E

Parameter	Processing plant		Experimental chiller	
	Calculated* from Z	Measured in PB	Calculated* from E	Measured in EB
Total solids	16	270	39	250
Suspended solids	1.0	57	1.6	33
Dissolved solids	14	220	37	220
BOD_5	0.50	82	0.40	67
Grease	0.49	41	0.48	25
Organic-N	0.0013	5.7	0.16	8.1
Ammonia-N	0.0016	0.43	1.8	1.1
Manganese (µg/1)	0.17	2.7	0.24	4.3

*Assuming 12% water uptake by carcass in the chiller, followed by its dilution with 1500 ml of distilled water in the measurement process. The values thus calculated from Z and E are obtained by multiplying their respective concentrations in Tables 3, 4, and 5 by 0.0942.

TABLE 13. ADDITIONAL COMPARISONS OF PHASE 2 ANALYSES OF CARCASSES (WASHINGS) IN PLANT AND EXPERIMENTAL CHILLERS WITH THEORETICAL CONCENTRATIONS CALCULATED FROM THEIR RESPECTIVE WATER SOURCES, Z AND E, AS WELL AS CHILLER WATERS, PC AND EC

	Processing plant			Experimental chiller		
	*Calculated from			*Calculated from		
Parameter	Z	PC	Found in PB	E	EC	Found in EB
	Concentrations in mg/l					
Surfactants	0.009	0.021	0.081	0.004	0.04	0.05
Calcium	3.8	3.7	5.3	5.1	5.5	5.1
Potassium	0.27	3.5	18	1.4	2.4	16
Sodium	0.70	7.5	27	2.8	8.9	24
Magnesium	0.23	0.31	0.57	0.29	0.28	0.52
Chloride	1.1	11	27	8.3	19	25
Nitrate	0.38	0.083	0.20	0.33	0.25	0.17
Sulfate	0.91	1.4	9.5	14	16	8.3
	Concentrations in µg/l					
Copper	3.9	4.4	15	3.7	2.8	18
Iron	1.8	4.5	22	5.4	3.8	30
Lead	2.0	5.2	28	2.2	2.1	30
Zinc	2.4	2.7	27	2.6	3.0	16

*Assuming 12% water uptake by carcass in the chiller, followed by its dilution with 1500 ml of distilled water in the measurement process. The values thus calculated from Z, E, PC, and EC are obtained by multiplying their respective concentrations in Tables 3, 5, 6 and 7 by 0.0942.

This analytical approach can be mad with greater assurance for the parameters shown in Table 13 since for these, the chiller water analyses are also available. Among all these macro and trace parameters, only the means for zinc were statistically different in the carcasses (PB and EB); yet at least 7 of the 12 were different between Z and E, as well as between PC and EC. All of the theoretical values calculated for the four trace constituents were considerably below those actually measured in the carcasses, both for PB and EB. Among the nine macro constituents, when comparing the concentrations calculated from Z, only calcium and nitrate were at levels close to those found in PB; and only calcium, magnesium, nitrate, and sulfate were comparable for E compared to EB. These same relationships apply when comparing the values calculated for PC to PB, and for EC to EB. Additionally for the latter, surfactants are also comparable.

As was the case for ammonia in Table 12, the "calculated" sulfates of Table 13 are about twice those actually found in EB, and this also could be explained if the actual uptake of water from the chiller was the more realistic 6 percent than the maximum 12 percent permitted. The fact that the values tabulated for calcium, magnesium, and possibly nitrate are relatively constant in all columns would indicate that there is probably no major transfer in either direction from carcass to chiller, either at the processing plant or in the experimental chiller. For many of the other constituents the gradual increase from calculated Z to PC, and then to that found in PB, and the comparable increase in the experimental chiller system would lead to the reasonable conclusion that there is a net flux or leaching from the carcasses to the chiller, rather than the reverse. That is, these constituents measured in the carcass washings are coming primarily from the carcasses themselves, rather than the chillers; at the same time they are contributing to the concentration levels in the chillers, and thereby increasing them compared to those in their respective water supplies, Z and E. Among all the constituents shown in Tables 12 and 13, perhaps only for ammonia and sulfate in Phase 2 is there some evidence to conclude that the transfer might have been from the water to the carcasses, at least for the experimental chiller system. And yet, even for these parameters the PB and EB mean concentrations were not statistically different, indicating that these uptakes, if they were indeed occurring in the experimental chiller, were not substantial.

ORGANIC CONSTITUENTS

A variety of measurements of gross organic parameters and specific organic chemicals have been performed during and subsequent to Phase 2. These include BOD_5, organic nitrogen, CCE (carbon chloroform extract), halogenated methanes, MBAS (surfactants), pesticides, total organic carbon (TOC), and specific organics extracted with methylene chloride and identified by gas chromatography-mass spectrometry (GC-MS).

Analyses were performed for nine pesticides, six of which are specified in the E.P.A. Interim Primary Drinking Water Regulations (endrin, lindane, methoxychlor, toxaphene, 2,4-D, and 2,4,5-T) and three others (chlordane, heptachlor, and heptachlor epoxide). Samples for pesticide analysis were collected on three separate days in a three-week period and

the results are reported in Table 14. These analyses were discontinued when all samples from the second and third days were negative. No positive values were obtained for the E or Z samples, only for A, the raw waste, and PB and PC, the carcasses and chiller water in the plant. Although some pesticides obviously are present in the carcasses, probably as a result of their known presence in the poultry feed, and do thereby contaminate the chiller water, they were not detected in the renovated water and cannot be considered a health risk were this water to be recycled into the poultry processing plant.

On four successive weeks in November and December 1976 volatile halogenated methanes were measured, the results being shown in Table 15. The only organic found by this technique at quantifiable levels in the renovated water, E, was chloroform, the maximum concentration being 3 micrograms per liter. In some of the treated well water samples, Z, chloroform was measured at concentrations of less than one microgram per liter, and one showed traces of carbon tetrachloride and dibromochloromethane. For reference, the mean chloroform concentration in finished U.S. public water supplies was reported to be 21 micrograms per liter in the National Organics Reconnaissance Survey (22). Although these renovated water samples are higher in chloroform compared to the treated well water, they are still very low compared to the concentrations found typically in public water supplies, and well below the 100 ppb (μg/l) limit proposed for total trihalomethanes by the EPA (26).

On two separate days subsequent to Phase 2 samples of E and Z water were collected, extracted by methylene chloride, concentrated by distillation and evaporation of the latter, and analyzed qualitatively by GC-MS. Although not confirmed by comparison with knowns, the organics presumptively identified are listed in Table 16. Another set of compounds was also found in both types of water, namely halogenated and hydrohalogenated derivatives of cyclohexene. However, research here and elsewhere has confirmed that this is an artifact resulting from contamination of the highly purified methylene chloride with cyclohexene. As is apparent, none of the compounds identified and listed in Table 16 are chlorinated. The most likely source of the fatty acids, at least in the renovated water, is the waste from the poultry, and, therefore, is of no direct concern. In the renovated water dioctyl and dibutyl phthalate were identified, but only the former in the treated well water. Both of these compounds are used as plasticizers and have been widely found in a variety of waters, including potable municipal supplies (20).

A summary of the total organic carbon (TOC) analyses of samples taken on five successive weeks in November and December 1976 is shown in Table 17. Each individual TOC value is a result of at least two measurements on the same sample, the TOC being the difference between the total carbon and the inorganic carbon analyses. Although the mean of the TOC values for the renovated water samples at 20.0 mg/l was higher than that of the treated well water, 14.5 mg/l, they could not be considered different statistically by the t-test at alpha 0.05. Nevertheless, it is apparent that there is an increased TOC load in the system, as judged by the mean concentrations in the first lagoon (L_1), 50.5 mg/l; the second lagoon (C' and C), 35-39 mg/l; and the microstrainer effluent (D), 39.8 mg/l. It appears that there is some substantial reduction in the flocculation-sedimentation basin, since its effluent (X) has a lower mean concentration of 22.4 mg/l, about equal

TABLE 14. PHASE 2 - PESTICIDE ANALYSIS

	Number of samples analyzed					
Date	A	E	Z	PB	PC	EB
2/26	2*	2	-	1*	1*	-
3/9	1	1	1	2	1	-
3/15	2	2	2	3	-	3

*Samples which contained positive values for the pesticides analyzed

	Pesticides Analyzed and Found (ug/l)				
Pesticide	Sensitivity Limit	Criterion** Value	Positive values A	PB	PC
Chlordane	0.2-1	3***	-	-	-
Endrin	0.1	0.2	0.1	0.1	-
Heptachlor	0.06	0.1***	0.15	0.42	0.09
Hept. Epoxide	0.1-0.2	0.1***	-	-	-
Lindane	0.06	4	0.06	0.14	0.08
Methoxychlor	0.5-1	100	-	-	-
Toxaphene	3-6	5	-	-	-
2,4-D	0.05-1	100	-	-	-
2,4,5-T	0.5-1	10	-	-	-

**E.P.A. National Interim Primary Drinking Water Regs., except for:
***Proposed as above, but not adopted

to that of E. The samples from the three untreated wells, Y_1, Y_2 and Y_3, are also relatively high in TOC, as is the river water (R) downstream from the effluent from the lagoon. Although the renovated water is relatively high in TOC, it can be concluded that a substantial portion of the organic matter responsible is already present in the well waters used as the water supply. In addition, any incremental organic load is primarily due to the organic matter from the chickens themselves, such as the fatty acids identified in Table 16. Also as discussed previously with reference to Table 12, the organic materials related to BOD and grease, as measured in the carcasses PB and EB, are much higher than the values that are calculated to be contributed

TABLE 15. HALOGENATED METHANE CONTENT OF SELECTED WATER SAMPLES TAKEN FROM NOVEMBER 16, 1976 TO DECEMBER 8, 1976, INCLUSIVE

Sample	Approximate chloroform concentration (ppb)				
	11/16/76		11/23/76	11/30/76	12/8/76
C-1				0	Trace
C-2				0	0
C'-1		0			
C'-2		0			
D-1			0		
D-2			0		
E-1	3*	2-3	2-3	Trace	Trace
E-2	1*	0	Trace	0	1-2
X-1			0	Trace	0
X-2			0	0	0
Z-1**	<1*	Trace	0	0	0
Z-2	<1*	0	-	0	0
Lagoon 1-1	<1*	0			
Lagoon 1-2		0			
River-1			0		
River-2			0		

*Denotes analysis performed by Calgon which acts as a means of comparing the technique of the University of Pittsburgh with that of an outside laboratory.

**This Z sample which was analyzed by Calgon showed traces of CCl_4 and $HCBr_2Cl$; none of the other samples analyzed by the laboratory showed the presence of any halogenated methanes other than chloroform.

TABLE 16. ORGANIC CHEMICALS IDENTIFIED QUALITATIVELY BY GC-MS ANALYSIS OF METHYLATED (WITH DIAZOMETHANE) METHYLENE CHLORIDE EXTRACTS OF WATER SAMPLES

Chemicals found*	Treated well water (Z)	Renovated water (E)
Dibutyl phthalate	NF	+
Dioctyl phthalate	+	+
Methylated normal fatty acids‡		
C-10	NF	+
C-11	NF	+
C-12	+	+
C-14	+	+
C-15	NF	+
C-16	+	+
C-18	+	+

*Present (+); not found (NF)

‡C-n refers to an "n" carbon fatty acid

TABLE 17. TOC ANALYSIS OF SELECTED SITES SUBSEQUENT TO PHASE 2 (ppm)

Sample	Number	Total carbon			Inorganic carbon			TOC		
		Range	$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.	Range	$\bar{X}$	S.D.
C'	2	34-36	35.0	1.4	0	0	0	34-36	35.0	1.4
C	4	35-45	39.0	7.3	0-0.5	0.25	0.4	34.5-45	38.8	4.9
D	2	35-61	48.0	18.4	0.5-16	8.25	10.9	34.5-45	39.8	7.4
E	8	15-45	22.0	5.4	0-14	2.00	4.9	15-31	20.0	5.1
L_1	2	50-55	52.5	3.5	1-3	2.00	0	49-52	50.5	2.2
R	2	16-18	17.0	1.4	0.5-1	0.50	0	17.5-19	16.5	1.2
X	6	20-29	22.7	1.8	0-1	0.25	0.4	19-28.5	22.4	3.2
Y_1	2	35-42	38.5	4.9	14.5-16	15.25	1.1	19-27.5	23.3	6.2
Y_2	2	20-25	22.5	3.5	14	14.00	0	6-11	8.5	3.6
Y_3	2	37-41	39.0	2.8	15.5	15.50	0	21.5-25.5	23.5	2.8
Z	10	6-42	20.5	13.8	0-16	6.10	7.2	6-26	14.5	7.0

from their respective water sources, Z and E. It is thus clear that the substantial exposures of the carcasses to organic matter in the chillers are mostly due to washings from carcasses previously moving through the counter-current flow of water.

Several carbon chloroform extract (CCE) measurements were made, both during Phase 2 and at other times. These measurements were done by the newer miniaturized two-day sampling technique (5), for which a standard had been proposed of 0.7 mg/l compared to the 0.2 value for the older technique in the 1962 PHS standards (28). In fact, it was shown in a comparison of the two techniques that the newer method measures about 6.7 times as much CCE as the older one (5). Over a period of approximately fifteen months, including the Phase 2 period, the CCE analyses on the renovated water (16 samples) shown in Table 18 averaged 0.6 mg/l, with a standard deviation of 0.4 mg/l. For comparison, a single measurement of the normally treated well water also had a CCE value of 0.6 mg/l. These results are consistent with the observation made above that, although apparently somewhat higher for E, there is no statistical difference between the TOC averages for Z and E samples.

MICROBIOLOGY AND RELATED

Residual Drug Assay

The purpose of the residual drug assay was to measure antibiotic activity in the poultry, wastewater, and renovated water that could result from the addition of such drugs in the feed of the poultry. It was ascertained for example, that bacitracin is added at concentrations of about 5 g per metric ton of feed.

Initially, samples were tested using Bacillus subtilis as the test organism and seed agar (antibiotic medium no. 1) as the test substrate. This procedure was, however, modified to enable the detection of bacitracin and sulfa drugs (incorporated in the feed of the chickens) by employing Bacillus cereus as the test organism and Mueller-Hinton agar as the test substrate, respectively. Only the samples collected on the last two sampling days in Phase 2 were tested by the modified procedure. Samples were tested on six sampling days in Phase 2 for wastewater, renovated water, treated well water, experimental and plant chiller water, and carcasses. All samples were negative. That is, zones of inhibition, indicating the presence of drugs, were not observed on the assay agar plates for any of the samples. The control antibiotic discs showed zones of inhibition. Diameters of the zones of inhibition ranged from 18 mm to 22 mm for 1 unit of penicillin-G per ml using Bacillus subtilis as the test organisms, and from 29 mm to 30 mm for 1 mg of streptomycin-sulfate per ml using Bacillus cereus as the test organism.

Subsequent to Phase 2 a variety of similar samples were tested using Sarcina lutea, which is more sensitive to bacitracin (19) than is Bacillus cereus. All such samples were similarly found to be negative. It may then be concluded that either the bacitracin is metabolized and, hence, not present in the carcass washings and wastewater, and/or this residual drug assay is not sufficiently sensitive.

TABLE 18. CARBON CHLOROFORM EXTRACT CONCENTRATIONS (CCE)

1976 week sampled	CCE mg/1	1977 week sampled	CCE mg/1
	Untreated well		
3/8	0.58	-	-
	Renovated water		
3/15[a]	0.96	4/14	0.71
3/29	1.2	6/16	0.42
4/19	1.5	6/28	0.24
5/18	0.18	6/30	0.35
6/9	0.59		
6/15	0.36		
6/23	0.44		
7/2	0.22		
7/16	0.61		
7/28	0.58 0.61		
11/15	0.52		

Renovated water - Arithmetic mean 0.59 mg/1

Standard deviation 0.36 mg/1

[a] stored water

Coliform, Fecal Coliform and Total Plate Count

The total coliform and fecal coliform results for the treated well water (Z) were negative on five of the six sampling days tested (10 samples) during Phase 2. The MPN results for these organisms as performed by the MHD Cumberland laboratory actually indicate that these were less than 2 organisms per 100 ml, and this will be cited here as negative. On one sampling day two of the Z samples were reported as each having 2.2 organisms per 100 ml for both total and fecal coliforms. This can occur occasionally and since it was a single occurrence, it is not of particular concern.

The renovated water (E) was analyzed for bacteria on eight days during and just prior to the Phase 2 period, 19 samples being taken. On six of the eight days the total and fecal coliform results were less than 2 organisms per 100 ml. On April 5 and 12 four E samples were analyzed. The reported total coliform ranged from 15 to >240 organisms per 100 ml, and the fecal coliform from 9 to >240. However, there is a strong likelihood that these E sample results were confused with those of EC, the experimental chiller water. The results for the latter for those two weeks were reported as negative for coliform and fecal coliform, a highly unlikely result compared to all other EC and PC coliform analyses which were >240.

The total bacterial plate count analyses on the samples described above indicated a maximum value of 25 organisms per ml for the Z samples and 15 for E, except for the four samples which are believed to be incorrect. For these latter four, the values ranged from 1 to 2,000. A quite reasonable total plate count for municipal water is 500 per ml.

For several months subsequent to Phase 2 samples of the renovated water were taken and analyzed for coliforms and standard plate counts. Of the 59 such coliform analyses, 55 were negative (MPN <2 per 100 ml). The four positive samples had MPN values of 3, 2.2, 3, and 8, respectively. Of the 54 samples analyzed for standard plate count, 28 were less than one per ml. The remaining 26 had a mean standard plate count of 3.46 per ml, with a standard deviation of 3.75.

In the earlier study of Clise (7), it was reported that for 352 samples analyzed for bacteria, counts were obtained consistently as follows: <3 coliform per 100 ml; and <1 fecal strep per 100 ml. From this earlier study, as well as that of Phase 2 and subsequently, it is clear that the renovated water has low bacterial counts. The many samples consistently low in bacteria also confirm the likelihood that the four high count samples in Phase 2 were a result of mislabeling of the samples, as discussed above. Also, as will be discussed below, no renovated water samples have yielded any enteric pathogens.

In addition, as emphasized throughout this report, in actual reuse the renovated water would receive additional full-scale water treatment, including disinfection with chlorine. It is thus apparent that in such reuse the 50/50 mixture of treated well and renovated water should be very low in bacterial contamination and unlikely in that respect to constitute a risk to human health.

Salmonella Isolations

One of the microbiological constituents studied was Salmonella because of its well-established occurrence in poultry and in wastewater from poultry processing plants. Initially, using SBG enrichment and BGA media, Salmonella organisms were isolated only from the untreated wastewater (A), the poultry carcasses (PB), and the plant chiller water (PC) during Phase 2. Table 19 summarizes the data on the samples taken and those that were positive. A total of 37 such isolates were identified as Salmonella and speciated as S. enteritidis. None were isolated from E (the renovated water), Z (the treated well water), the lagoons, C or C' (the unchlorinated and chlorinated effluents from lagoon 2 respectively), EC or EB (the experimental chiller water and carcasses exposed therein, respectively), nor from any other point in the water renovation system itself.

Quantitation of Salmonella by the MPN method was attempted for the samples collected during Phase 2 using SBG as enrichment. However, even though each morphologically different type of oxidase-negative, pink-colored colony was picked from each BGA plate and identified, the majority of these colonies were non-glucose-fermenters (i.e., false positive organisms), rather than Salmonella. The large numbers of these false positive organisms made the attempts to quantitate Salmonella by the MPN index using SBG unreliable and irreproducible.

Another broth recommended by others (1, 9, 11) to support the growth of Salmonella is TET enrichment. For inoculated water samples in our laboratory MPN indices for S. typhimurium were significantly higher at the 95% confidence level in the TET broth than in the SBG enrichment. Subsequent to Phase 2 TET was, therefore, used to attempt to quantitate Salmonella. For such water samples collected from the poultry plant the incidence of false positive organisms was less than 50 percent. MPN indices of Salmonella ranged from 0.04 to 7.30. For 14 of 17 Salmonella positive samples (82%), MPN indices were <1 Salmonella per 100 ml, indicating relatively low recovery. Although the still relatively high percentage of false positives precluded accepting these MPN results with confidence, the sensitivity of the TET enrichment was greater than that of SBG, as judged both by the laboratory inoculation comparison and the fact that more samples were positive. Thus, in contrast to the Phase 2 results with SBG shown in Table 19, using TET all 6 of the wastewater (A) samples were positive, as were 11 of 15 lagoon samples (in comparison to 0 of 16 in Phase 2 with SBG). As would be expected, all the finished well water (Z) or renovated water (E) samples were negative.

Salmonellae were isolated from the untreated wastewater and the first lagoon, using BGA both with and without sodium sulfadiazine. However, they were isolated from the second lagoon only on the medium supplemented with sodium sulfadiazine. False positive organisms were never isolated from the BGA medium containing sodium sulfadiazine. In contrast, 46 to 77% of the organisms isolated from BGA not supplemented with sodium sulfadiazine were identified as false positive organisms. These data indicate that sodium sulfadiazine was effectively inhibiting the false positive organisms from growing on the BGA plates.

TABLE 19. SALMONELLA ISOLATIONS IN PHASE 2 AT VARIOUS SAMPLE SITES USING SELENITE BRILLIANT GREEN ENRICHMENT AND BRILLIANT GREEN AGAR

Type of sample	No. of weeks sampled	Total no. of samples	Number positive for Salmonella*
Carcass			
Plant (PB)	4	13	5
Experimental (EB)	4	14	0
Chiller			
Plant (PC)	3	3	1
Experimental (EC)	3	3	0
Wastewater			
Raw (A)	6	12	7
Lagoon (various sites)	3	16	0
Finished Water			
Well (Z)	3	12	0
Renovated (E)	7	26	0

*In each case identified by serotyping as Salmonella enteritidis, either group B or C_2.

MPN indices in 6 out of the 10 Salmonella positive samples were not significantly different at the 95% confidence level using the BGA with and without sodium sulfadiazine. The other four samples positive for Salmonella had significantly higher MPN indices on the BGA medium with sodium sulfadiazine. It is likely that these higher MPN indices can be attributed to the inhibition of the false positive organisms by sodium sulfadiazine, and thus the unmasking of the Salmonella colonies of the BGA plates. These results indicate that BGA medium containing sodium sulfadiazine is an effective plating medium both for supporting the growth of Salmonella organisms and inhibiting the growth of false positives. Although more such data are

required to prove this conclusively, it appears that this medium is effective when used in the MF technique to determine MPN indices of Salmonella in water and wastewater samples collected at poultry processing plants.

A summary of the results of the MPN indices for water and wastewater samples using TET enrichment and BGA with sodium sulfadiazine is shown in Table 20. Samples were taken on four dates subsequent to Phase 2, and none were positive in the renovated (E), treated well (Z), or chlorinated lagoon water (C). All of these latter samples had relatively large filtered volumes ranging from 500 to 3000 ml. Although the MPN indices in Lagoon 1 were not higher in each instance than those in the wastewater, there was a general reduction from the wastewater to the unchlorinated effluent from Lagoon 2. These results, along with those for the chlorinated lagoon effluent and renovated water samples, both in Tables 19 and 20, indicate that protection against Salmonella is highly effective in the overall renovation system.

Newcastle Disease Virus (NDV)

Although it is unlikely that viruses would survive the entensive chlorination in the wastewater, renovation, and water treatment systems, it was nevertheless considered important to have some evaluation of their possible presence and survivability. In the earlier study of the renovated water at the Sterling plant, Clise reported (7) that nine five-gallon composite samples of the renovated water were examined for human enteric viruses and found to be negative.

Because human wastes at the Sterling plant are segregated and not emitted to the lagoons receiving wastewater from the poultry processing operations, it is more likely that an avian virus could enter the renovation cycle. No such viral diseases are known to be transmitted to humans by the gastro-intestinal route. Nevertheless, it was decided initially to analyze carcass, wastewater and renovated water samples for such an avian virus likely to be present, in an attempt to assess the efficiency of the renovation process in removing viruses.

Newcastle disease virus (NDV) was chosen. It produces an infectious, highly contagious respiratory infection, mainly in chickens and turkeys, although other poultry and man can contract the disease from infected birds (3, 12, 16). Newcastle disease (or avian pneumoencephalitis) is characterized in birds by pneumonic and neurologic symptoms. However, in humans it is manifested as conjunctivitis without corneal involvement, and usually occurs only in poultry handlers and laboratory workers. Transmission (both bird-to-bird and bird-to-man) of the virus is accomplished either through the respiratory secretions or through the exudates, excreta and offal of infected birds. The incidence of Newcastle disease in chickens is higher in the fall and winter months, and the disease tends to disappear with warmer weather. Birds recovering from infection can harbor and eliminate NDV via the respiratory and gastro-intestinal tracts from 2 to 4 months. Reinfection of chickens can occur with this virus. In order to prevent transmission of this disease within a flock, the chickens are inoculated intranasally with an attenuated, live NDV vaccine. Thus it could be detectable in the slaughtered carcasses or the wastewater treatment system.

TABLE 20. MPN INDICES OF SALMONELLA FOR WATER AND WASTEWATER SAMPLES WITH TET ENRICHMENT USING BGA WITH SODIUM SULFADIAZINE, ALL SUBSEQUENT TO PHASE 2

Sample date	MPN index per 100 ml		
	Wastewater (A)	Lagoon 1, center (L-1-M)	Lagoon 2 effluent, unchlorinated (C)
11/11	4.25 3.71	-	-
11/24	0.28	0.07	-
12/1	21.6 40.9	-	-
12/8	1.10 0.18	0.29	0.04

Note
In addition, the following water samples on the indicated dates (and the number of samples in parentheses) were negative for Salmonella:
Renovated (E): 11/17 (4); 12/1 (2); 12/8 (1).
Treated well (Z): 11/11 (1); 12/1 (2).
Chlorinated lagoon (C): 12/8 (1)

NDV is a paramyxovirus that ranges in size from 70 to 120 mm. It is somewhat resistant to adverse environmental conditions, e.g., pH (stable at pH 2 to 12), temperature (stable at $\leq 50^{\circ}C$), light and moisture. Consequently, water can serve as a vehicle for transmission of the virus. NDV is capable of hemagglutinating chicken, guinea pig or human type O red blood cells. In the laboratory it is grown primarily in eggs by inoculation of the allantoic cavity; however, it has been grown also in primary chick embryo (CE) cells. Although NDV has also been grown in continuous primate cell cultures, e.g., HeLa and monkey kidney, a lower titer of virus is produced in the primate cells than in CE cells. The cytopathic effect (CPE) produced by NDV in cell cultures is characterized by syncytium or giant cell formation. Infected cultures can also show hemadsorption when guinea pig or chicken red blood cells are added.

NDV in Water and Carcass Samples--
Initially during Phase 2 attempts were made to detect NDV in the water

and carcass samples by inoculating chick embryo cells and using a plaque assay. However, other avian viruses that are capable of growing on primary chick embryo cells and forming plaques could have been detected in the samples. Several such samples collected on one day were inoculated on complete monolayers of primary chick embryo cells. After 4 days of incubation, cells were stained for plaques. Plaques were not observed on any of the cells inoculated with the water and carcass samples. The positive control, NDV at the 10^{-4} dilution, produced too many plaques to count on the chick embryo cells and thus had a titer of $\geq 10^{6}$ PFU/ml.

Since avian viruses were not detected in any of the water and carcass samples by inoculating chick embryo cells, tissue specimens were subsequently taken for analysis for the presence of avian viruses by a hemagglutination test. The isolation and identification of NDV was attempted, since the chickens were inoculated intranasally with an attenuated, live NDV vaccine. If, however, it could not be isolated in the slaughtered chickens, it is unlikely that it would be detected in the wastewater or renovation system.

Tissue extracts were prepared from 5 spleens, 5 livers and 5 lungs from chickens obtained from the Sterling plant, and inoculated into the allantoic cavity of eggs to permit avian viruses (if present in these tissues) to replicate to a high titer. Serial 2-fold dilutions (to the 1:2048 dilution) of the allantoic fluids were prepared and employed in hemagglutination tests. No agglutination of the chicken red blood cells was observed with any of the 15 tissue extracts. The NDV positive control produced hemagglutination titers of 4. The influenza-A virus controls produced hemagglutination titers of 64 and 16. The decrease in the hemagglutination titer of influenza-A virus was attributed to the virus undergoing one freeze-thaw between the two tests which lowered the virus titer.

Although extensive testing was not performed, neither NDV nor other avian viruses that would have been capable of growing on primary chick embryo cells were thus found in the water, wastewater, or carcass samples, and NDV was not detected in the tissue extracts. Since NDV is the most likely avian virus of possible concern, however remote, in the renovation system, attempts to isolate and identify viruses at the Sterling plant were discontinued.

NDV Survival in Lagoon Water--

Since NDV could not be detected in the carcasses, wastewater, nor at any point in the wastewater treatment or renovation system, it was decided to study its survival in lagoon water in the laboratory. The purpose was to obtain an indication of the effectiveness of one important stage in the overall treatment process not specifically designed to remove viruses, but which would be expected to have an effect. The disinfection processes themselves would certainly further reduce the concentrations of any viruses. Although the stability of NDV in the wastewater treatment lagoons would not necessarily be indicative of the behavior of other viruses, if it were relatively unaffected by the lagoon, then this would indicate that disinfection with chlorine and other stages of treatment in the renovation system might have to be relied on to be confident of virus removal.

The laboratory NDV survival experiments measured the possible effects of light and temperature in lagoon, filtered lagoon, and distilled waters. The flasks, inoculated with NDV, were exposed either to white fluorescent light or kept in the dark, in each instance either at 25°C or 7°C. The flasks were gently agitated and samples taken daily for several days and assayed for NDV.

The initial results showed some differences, but not consistent, in survivability in the light and dark, and a considerably higher rate of die-off in lagoon water at 25°C compared to 7°C. More extensive experiments confirmed that there is a temperature effect, although not quite as large, and typical results are shown in Figure 2 and Table 21. In the former the survival as a function of time is shown in the three types of water in light and at 7°C only. The one-day titer for the lagoon water (LW) in Figure 2 is off scale because it is a few percent higher than the initial 100 percent. This is likely due to analytical variability. Table 21 summarizes only the 5-day survival data.

Although the survival curves shown in Figure 2 are not smooth, and the differences due to the experimental variables not consistent, some conclusions, although not necessarily firm, can be made. There is no consistent difference between the results in the light and dark, or in the filtered and unfiltered lagoon waters. The survival, at least over the full five days, is generally greater in distilled water than in either of the lagoon waters. And finally, the survival is consistently greater, although of varying relative magnitudes, at 7°C than at 25°C.

The time for 50 percent die-off in the lagoon waters varied from about 1 to 3 days at 25°C, and from 2½ to 5 days at 7°C. The survival curves, plotted semi-logarithmically in Figure 2, are not generally linear and may not follow first-order kinetics. Nevertheless, with the assumption that they do, one can estimate NDV survival in the lagoon system. For the approximately 15-day residence time in the two lagoons, the 5-day half-time would correspond to a survival of 0.125, and the 1-day half-time to 0.00003. To the extent that these laboratory experiments simulate accurately the NDV behavior in the lagoons, it is apparent that there is significant die-off, and that the disinfection and other stages in the water renovation process may not have to be relied upon as the only protection against possible virus contamination in the reuse of wastewater at the Sterling plant.

STEADY-STATE CONSIDERATIONS

An important question for any wastewater recycling process is the extent to which the concentrations of waste constituents can build up. The steady-state mass balance equations for such possible build-ups have been developed for the Sterling Plant system in Appendix B, which also considers in some detail the extent to which the Phase 2 flow regime, without actual recycle, can be used to predict the steady-state concentrations that would occur with recycle and reuse of the renovated water. As long as the process does not involve a closed loop (no blow-down or partial diversion to receiving waters), or there is some removal in treatment, mass balance considerations indicate that a steady-state, rather than an infinite build-up will occur. In the

TABLE 21. SUMMARY OF NDV LABORATORY SURVIVAL EXPERIMENT, PERCENT SURVIVING AFTER 5 DAYS

Water type	Percent survival			
	7°C		25°C	
	Light	Dark	Light	Dark
Lagoon	26	15	7	8
Lagoon, filtered	13	18	0	17
Distilled	49	32	17	22

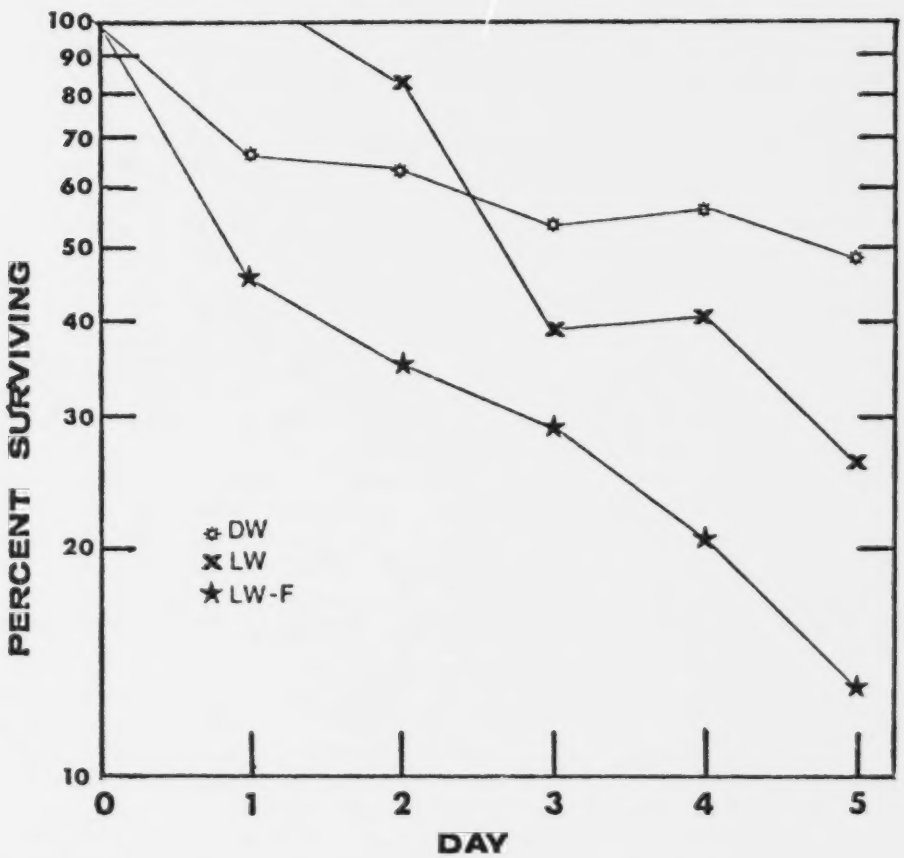


Figure 2. NDV survival at 7°C in light in laboratory study. (DW: distilled water; LW: lagoon water: LW-F: lagoon water, filtered)

Sterling Plant partial removal in two modes can occur. The planned 50/50 mixing will result in at least half of the waste constituents generated in any one cycle being emitted to receiving waters. The lagoon treatment and the renovation system can also reduce the build-up. For this combination of removals, and using mass balance, one can estimate the steady-state concentration in the 50/50 renovated water, C_s, of the daily waste load, A, of a parameter emitted from the plant. Such a relationship was developed in Appendix B (Equation 5) and can be written as:

$$C_s = (A/V)(bc)/(2-bc) \quad (1)$$

with V the daily volume of water used, and the fractional removals of the constituent in question being (1-b) in the lagoons and (1-c) in the renovation system. The ratio A/V is also simply the concentration of a constituent in the waste, C_A. The fractional concentration remaining in the renovated water (prior to mixing with the well water) compared to the average concentration in all waters discharging into the first lagoon is bc. It should be noted that Equation 1 assumes that the concentration of the constituent emitted as a waste from the poultry processing plant is zero in the well water supply, and is not added or removed in the normal water treatment received by the 50/50 mixture of renovated and well water.

Aside from being able to calculate the steady-state concentration in the water supply in actual recycle using Equation 1, the question arises also as to whether the steady-state recycle of Phase 2 is a reasonable simulation of actual recycle into the poultry plant. As shown in Figure 1, in Phase 2 the renovated water effluent was recycled into the first aerated lagoon, whereas with reuse it would be mixed 50/50 with the untreated well water, the mixture then to receive normal treatment. Also in Phase 2 the flow through the lagoons was about one-third higher than it would be in actual recycle, with the flow through the renovation system being about one-third of that through the plant (compared to one-half in actual recycle). This results in a higher dilution of waste constituents emitted to the lagoon and cycled through the renovation system in Phase 2. However, in actual recycle the 50/50 mixing with well water will tend to reduce its steady-state concentration compared to that in Phase 2. Combining these factors in a mass balance calculation permits a comparison of the steady-state concentrations likely to be achieved in actual recycle (after 50/50 mixing with well water) to that of the renovated water in Phase 2, the ratio of these two being r_s. In Appendix B Equation 20 expresses such a relationship for this ratio r_s as follows:

$$r_s = (1/3)(4-bc)/(2-bc) \quad (2)$$

Thus, this ratio is highly dependent on the fractional removals in the lagoon and renovation systems. With high degrees of removal (bc approaching zero) r_s will approach 2/3. However, with very low fractional removal (bc not much less than unity), the ratio will approach 1.0. Thus, one can predict that, as a first approximation, the steady-state concentrations of the 50/50 renovated water mixture in actual recycle will, depending on the waste constituent, be 0.67 to 1.0 times those observed in the renovated effluent

in Phase 2. Other factors will modify the actual result, such as the fact that the 50/50 mixture in actual recycle will undergo further treatment, or the well water itself can contain a significant concentration of the constituent in question. Nevertheless, the Phase 2 study may be taken as a reasonable predictor for full recycle and reuse.

One could estimate bc for a given parameter from a knowledge of its concentration in A and E during Phase 2, and this would be simply C_E/C_A. However, because the flow regime in the lagoons in Phase 2 is different than it would be in full recycle, this estimate could be incorrect and, therefore, misleading. In Phase 2 the actual volume of flow into and through the lagoons consists of three parts of waste A from the poultry plant and one part of renovated water E, so that the effective concentration of a constituent discharged into the first lagoon is $(3C_A + C_E)/4$. Thus, during Phase 2 the actual fractional concentration of a constituent remaining in the renovated water after moving first through the lagoons, and then the renovation system, is simply C_E divided by this expression for the effective concentration, namely:

$$bc = 4C_E/(3C_A + C_E) \qquad (3)$$

A useful constituent to demonstrate these relationships is potassium, since, as shown in the Phase 2 results of Table 3, it is quite low in the treated well water, Z, it is found in much higher concentrations in A, and is then significantly reduced in concentration as it appears in the renovated water, E. For the purposes of this calculation we will neglect its concentration in Z, and take C_A and C_E as 32 and 15 mg/l, respectively. Using Equation 3, the calculated value of bc is 0.54. In contrast, the approximate estimate of bc, simply using C_E/C_A is 0.47, about 13 percent lower than the correct value. Having calculated bc for potassium, one could then use Equation 1 to calculate its expected steady-state concentration, after 50/50 mixture, in actual reuse, and this value, C_S, is 11.8 mg/l. Alternatively one can use Equation 2 to calculate r_S, the ratio of potassium expected in the 50/50 mixture in actual reuse compared to that in E in Phase 2. Again using the 0.54 value for bc, r_S is calculated to be 0.79. As a check on the self-consistency of these equations, C_S, calculated from Equation 1, divided by C_E is 11.8/15 which equals 0.79.

These calculations can be modified readily, as discussed in Appendix B, to meet a variety of circumstances, even for such a constituent as sulfate, which, as shown in Table 3, seems to be generated in large concentrations within the renovation system itself probably from alum. To the extent that this is so, one can still estimate C_S for sulfate. Neglecting the contributions of sulfate from the treated well water and the poultry processing plant, and assuming that there is no reduction of its concentration in the lagoons, this is equivalent to the statement that bc equals unity. Also, if 150 mg/l sulfate were to be generated in the renovation system in actual reuse, as we are assuming it is in Phase 2, this would result in C_S also being 150 mg/l, since this concentration would ultimately return to the renovation system, the total leaving it then becoming 300 mg/l. This then would reduce to 150 mg/l (C_S) after 50/50 mixing with well water. Thus,

even where constituents are generated within the renovation system because of chemical addition, and with no fractional removal elsewhere, there would be only a finite build-up, and a predictable one.

Another important question concerning the Phase 2 study and actual recycle is the rapidity with which steady-state is achieved. As discussed in Appendix B, this is dependent principally upon the fractional removal in the lagoon and renovation system, and, hence, bc. Using Equation 15 of Appendix B, one can calculate the concentration ratio in the 50/50 mixture achieved after "n" cycles, C_n, compared to that in the steady-state, C_s, using the flow regime contemplated in actual reuse. Doing this for 3 cycles (n=3), one obtains values of C_3/C_s equal to 0.91 and 0.998 for bc equal to 0.9 and 0.25, respectively. It is, therefore, apparent that, even over a wide range of fractional removals, steady-state is approached rather rapidly. Although this calculation does not apply exactly to Phase 2, which is a different flow regime, it can be used as a first approximation for it. In the Sterling system the residence time in the lagoons determines the time for a cycle. This is normally approximately two to three weeks. Because of the increased flow through the lagoons in Phase 2, this is probably reduced by about 25 percent, perhaps to two weeks. Phase 2 itself covered a period of seven weeks, with a two-week preliminary sampling period. In addition, the preceding Phase 1 period involved several additional weeks with the same flow regime. It can, therefore, be concluded that the Phase 2 study was in steady-state, and its results can be used to estimate concentrations in the renovated water in actual reuse.

SECTION 7

DISCUSSION

The focus of this discussion will be whether the quality of the renovated water, E, is sufficient to justify its reuse in processing poultry at the Sterling plant, without risking the health of the consumers. It should be emphasized that, prior to actual use in the plant, the renovated water will be mixed 50/50 with the untreated well water, then receive the normal water treatment that is currently utilized for it, including two stages of chlorination in addition to that received by the lagoon and renovation system effluents. Since the chiller is probably the single most important water exposure of the birds in the processing plant, it is necessary to consider not only whether there is a possible build-up of contaminants in the renovation system, but also whether such concentrations cause a substantial transfer of such materials to the "dressed" birds or carcasses; and, if so, does this constitute a health hazard to the consumers of the chickens. Thus, not only the quality of the renovated water, but also its intended use must be considered in making the judgment whether to proceed to a trial period of reuse and, ultimately, to such reuse on a permanent basis.

Before reviewing the findings that would help answer these questions, it is important to note that the analysis of the mass-balance steady-state relationships indicates that the flow regime in Phase 2 is such that the concentrations of constituents measured in the renovated water, E, can be regarded as reasonable predictors of those that can be expected in the 50/50 treated mixture in the steady-state during actual recycle. For a given parameter the exact ratio, r_s, between these concentrations in such reuse compared to E in Phase 2 is dependent on several factors, not all of which have been determined for every constituent. The likely maximum values of r_s can be estimated, and it is unlikely that any of the constituents studied could build up to hazardous and unpredictable levels. Certainly the careful monitoring program of the proposed trial period of reuse would quickly discover this unlikely event. Even if there were no removal in the lagoons or renovation system, the maximum steady-state concentration, C_s, in the planned 50/50 reuse flow regime (with the waste from the processing plant itself being the only source of a given constituent) would simply be equal to its concentration in the plant wastewater without any recycling. Similarly, where either the well water or renovation system were to be unique sources, C_s would equal its respective concentration in the absence of any recycling. This apparent lack of build-up is in fact due to the 50/50 dilution flow regime. It indicates, for example, that if a contaminant were to get into the well water source and not be removed subsequently at any stage of treatment, it would never reach a concentration in the renovated 50/50 mixture,

C_s, higher than that in the untreated well water itself. This would similarly apply to concentrations generated in the water renovation system, such as due to the addition of chemicals like alum. This possible situation, with sulfate as the example, was discussed in Section 6.

It was also shown that although dependent on the removal factors. steady-state is approached very rapidly. For the practical purpose of using Phase 2 as a predictor for reuse, and using the Phase 2 two-week residence time in the lagoon system as the determinant of the time for a single cycle, it was judged that steady-state was achieved.

The quality of the renovated water itself, and its comparison with the treated well water and any appropriate water quality standards are of immediate interest. Although the renovated water is not intended to be utilized as a drinking water supply, it is required by the U.S. Department of Agriculture to be potable and to be so certified by the appropriate governmental agency. Prior to June 1977 the standards most commonly used for this purpose in the United States were the 1962 U.S. Public Health Service (PHS) Drinking Water Standards (28), which include both primary standards with mandatory, health related constituent limits, and secondary ones with recommended limits, principally related to esthetic concerns. The newer U.S. Environmental Protection Agency (EPA) Interim Primary Drinking Water Regulations, effective in June 1977 (25), and the proposed EPA Interim Secondary Drinking Water Regulations (26) are very similar to, but not identical to the PHS standards. These various standards address many constituents measured in this study and both the treated well water and the renovated water quality were compared to them.

With regard to such parameters the Phase 2 results indicate that in almost every instance both the renovated (E) and treated well (Z) waters, as shown in Tables 3 and 5, were well below the EPA and PHS criterion limits. The newer EPA turbidity limit of one unit was frequently exceeded in both of these waters during Phase 2, but their respective maximum values were well below 5 units (the PHS limit), which is permitted by the EPA if there is no interference with disinfection or microbiological determinations. The fact that there were acceptably low bacterial levels indicates then that the turbidity levels are also acceptable. It should also be noted that there was no statistical difference between the mean values for turbidity in Z and E water. Sulfate values in the renovated water were high, but only one measurement marginally exceeded the recommended (not mandatory) secondary criterion of 250 mg/l. The only other marginally high constituent was lead which, however, had almost identical mean concentrations in the Z and E waters, and both had maximum concentrations at the EPA criterion limit of 50 µg/l. Because the mean concentrations of both waters were about one-half of this limit, one can conclude that the lead levels cannot be regarded as hazardous. In any event the much higher lead levels in the chiller water in the plant, and in its effluent, along with the washings from the carcasses, indicate that neither the Z nor the E waters in Phase 2 constituted a substantial source of lead to the processed birds. The renovated water quality in the previous study (7), shown in Table 10, also met these various standards with one or two exceptions. The turbidity was higher than in Phase 2, but still acceptable on the basis discussed above. The color of the water was

very much higher than in Phase 2, and well above the proposed EPA secondary limits. Thus the Phase 2 results constitute an important improvement. The iron levels in the renovated water were also much higher in the earlier study, the mean concentration of 0.27 mg/l approaching the EPA secondary limit of 0.3 mg/l. However, the Phase 2 mean concentration of 0.057 mg/l, although higher than 0.019 mg/l in Z, also represents a significant improvement. Also, as with lead, the evaluation of iron levels in the carcasses showed that there were no substantial contributions from the Z and E waters. One can conclude, therefore, that for those macro and trace inorganic chemicals, as well as other water characteristics for which drinking water standards are available, the renovated water in the Phase 2 study, as well as the treated well water, was of acceptable quality.

In addition to those constituents in the renovated and treated well waters addressed by the standards previously discussed, other measured inorganic constituents and waste parameters in many cases, as would be expected, were substantially higher in the renovated water as compared to the treated well water in Phase 2. Thus, the macro inorganic ions, other than nitrate, all increased to the point of raising the total dissolved solids level in E to an average concentration of 389 mg/l (below the 500 mg/l EPA secondary guideline) compared to 143 in Z. However, this increase, as shown in Table 3, can be attributed essentially to the macro inorganic cations and anions, and these are of no human health significance at the levels encountered. It is, therefore, unlikely that any unidentified constituents are making substantial contributions to this increased quantity of total dissolved solids in E. As noted in Section 6, the high average ammonia concentrations, 19 mg/l in E during Phase 2, were substantially lower at 5.1 mg/l subsequently. Although the ammonia concentrations in the carcasses were somewhat higher for those exposed to E versus Z, and the data suggest that indeed the ammonia in E was probably responsible, the mean concentrations in PB versus EB were not significantly different. It is highly unlikely that these levels constitute a health hazard.

The organic chemical quality of the renovated water at the Sterling plant remains perhaps the greatest area of possible concern, as it does for the municipal water supply systems of the U.S., principally because of the recent advances in the technology to identify and quantify trace organic chemicals at very low concentrations, as well as their possible influence on chronic disease in humans. The measured gross organic parameters, namely BOD, CCE, TOC, and organic nitrogen, are of interest as indicators of specific organic constituents. The much higher organic nitrogen in the renovated water most probably reflects the proteinaceous material and its breakdown products from the poultry, but only those constituents that are not readily biodegradable, since the BOD values for the renovated and treated well waters were quite comparable. The mean BOD value of 3.4 mg/l for the renovated water is not untypical of many raw surface waters that are used for municipal water supplies, such as Minneapolis and St. Cloud, Minnesota (17).

The mean TOC values for the renovated and treated well water, 20 and 14 mg/l, respectively, are high, but not uniquely so. The statewide average for the major Minnesota rivers is 20 mg/l, with several large municipal supplies utilizing them as raw water supplies (17). Similarly, most of the

larger rivers there have concentrations of 15 to 30 mg/1. In a study of 80 municipal water supply systems of the U.S., non-volatile TOC concentrations as high as 19 mg/1 were measured in the raw water and 12 mg/1 in the finished water (22). About 98 percent of the latter were less than 5 mg/1. This indicates that, in terms of a finished water supply, the renovated water TOC values are undoubtedly high. However, after mixing 50/50 with the raw well water and then full-scale treatment, the final mixture should not be significantly different in TOC than the currently used treated well water.

The CCE measurements of the finished water show considerable variability. The mean concentration in E over a 15 month period, 0.59 mg/1, is about equal to the single value of 0.58 mg/1 measured in Z. However, time variations, seasonal or otherwise, are also common in finished municipal water supplies (5). Similarly, concentrations of 1.0 mg/1 of CCE are not uncommon in finished water supplies, and concentrations considerably higher than this have been found in rivers. For reference, 0.7 mg/1 has been proposed recently (5) as a criterion value for public water supplies. Finally, it should be noted that the gross organic parameters BOD and organic nitrogen had very similar concentrations in the carcasses exposed, respectively, to the normally treated well water and the renovated water. Also in both cases the calculated contributions of these constituents to the levels actually found in the carcasses were very small indeed, namely 2 percent or less. Thus, there is no significant detectable increase in gross organic contamination of the carcasses or the renovated water itself in Phase 2, compared to the treated well water and the carcasses exposed to it.

Several specific organic chemicals have been identified, and some quantified in this study. Pesticides were not found in either the renovated or treated well water. Surfactants in the former were well below criterion levels. Several fatty acids were found in the renovated water, but also in the treated well water. In any event, these constitute no human hazard. The maximum concentration of the only halogenated methane found regularly in the renovated water, chloroform, was three micrograms per liter, well below the approximate median value of 20 found in the EPA National Organic Reconnaissance Survey of U.S. public water supplies (22). Two phthalates were found in the renovated water, and one of these in the treated well water. As noted previously, both of these, widely used as plasticizers, have been found in potable U.S. municipal water supplies, as well as many natural waters.

It should be emphasized, however, that there have not been extensive analyses of specific trace organics utilizing a variety of concentration, separation and detection methodologies. Nevertheless, the measurements that were performed on the water did not uncover any concentrations of potentially toxic chemicals, at least at concentrations likely or known to cause human disease. The specifically identified organic chemicals arising from this waste renovation process do not constitute a human hazard were this water to be used as contemplated in full recycle. One might nevertheless raise the question of the possible health hazard from organics not yet identified. It is unlikely that, in terms of the reuse of this water for processing poultry, such organics would be hazardous, since they arise primarily and originally from the poultry wastes and are likely to be only

natural materials and their degradation products. A possible question concerns the reaction of chlorine with these materials to form hazardous by-products. This concern is difficult to address. It should be pointed out, however, that chlorination is practiced in certain food processing, and has not been associated with any ill effects in humans.

Before leaving the question of the possible impact of various chemicals on the carcasses, the findings on the possible relationships between their analyses in Phase 2 relative to their respective water supplies and chiller waters should be reviewed. Among all the inorganic constituents and physical parameters measured, there were in almost every case no statistical differences between carcasses processed in the plant and experimental chillers. Zinc was higher in the plant, and ammonia in the experimental chillers. Furthermore, only for a few inorganics, and none of those with health related criterion limits, did there seem to be any possible impact on the carcass analyses. Such comparisons indicate, for the most part, that the movement of such constituents in the chiller system is from the carcasses to the chiller, rather than vice versa. This is certainly the reason for their presence in the waste effluent from the plant at substantially higher concentrations than in the treated well water. This additional perspective on the chemical water quality must be emphasized because, in the last analysis, the critical question is indeed, not whether the water meets particular standards, even though it may and in this instance does, but whether it has any impact on the quality of the processed chickens and the health of the consumer. A careful analysis of the Phase 2 data and their interpretation indicates that there is no basis for concern about the quantities of specific chemicals identified in the carcasses. The only remaining uncertainty, however small, is the trace levels of chlorinated organics, so far undetected, that might be formed in the renovation system. However, this could be ascertained with additional study, either prior to or during a Phase 3 trial period of reuse.

The low bacterial counts in the renovated water, as well as the measurements showing the absence of specific pathogens such as Salmonella, demonstrate that bacteria from the water supply do not constitute a risk. The presence of a variety of such bacteria in the plant chiller water or carcasses is certainly not unusual, and they have been shown to build-up rapidly in the water as the poultry contact it (10). Newcastle Disease Virus that might be expected in the poultry or wastewater could not be found in either. The laboratory die-off experiments with this virus using lagoon water from the Sterling plant indicated that one can expect substantial viral removals in the aerated lagoon system. In view of the approximately two-week's detention time in the aerated lagoons and the nature of the disinfection processes subsequent to them, which involve two stages of chlorination, this excellent microbiological quality is to be expected. With actual recycle into the plant, this high quality and the additional treatment, including disinfection, would insure, with a high degree of certainty, that there would be no danger from pathogenic organisms in the reuse of this renovated water.

An evaluation of many of these results was performed immediately following completion of Phase 2 by a committee constituted to do so and make a recommendation about proceeding to Phase 3, a three-month trial period of

full recycling through the plant and water reuse. That committee did not at the time have access to some measurements of organic water quality which were done subsequent to Phase 2, including most of the CCE analyses, and all of the TOC and specific organics, other than pesticides. The committee recommended that there was no significant risk in proceeding to Phase 3. The additional data on organic chemical quality should not modify that judgment.

Nevertheless, the final decision to proceed to Phase 3 was not and has not been made as of this writing. The delay arose because of the requirement of the Department of Agriculture that the water to be reused in the plant be designated as potable. There are differing opinions as to whether it could be so regarded. The concerns center around two areas. First, is the chemical and microbiological quality of the renovated water sufficient to meet criteria of potability? In terms of meeting constituent limits specified in drinking water standards or regulations, the answer is yes. The gross organic load is high, but not much more so than the normally treated well water. Nevertheless, a certification of potability has been made by the legally authorized agency, the State of Maryland.

The second area of concern related to potability is the nature of the raw water source. A long-standing concept, as stated in the 1962 Public Health Service Drinking Water Standards (28), is the following:

> "The water supply should be obtained from the most desirable source which is feasible, and effort should be made to prevent or control pollution of the source. If the source is not adequately protected by natural means, the supply shall be adequately protected by treatment."

In terms of the intended goal of this water renovation system, namely the augmentation of the limited non-community well water source for the Sterling plant, other possible available sources should be considered, using the above concept. The local community, Oakland, will not and cannot provide additional water to the Sterling plant. A detailed analysis on the specific current water usages at Oakland, projected population growth, and water treatment plant capacities, indicate that any substantial commitment of water to the poultry processing plant could not be met (14). The only other possible source is the Little Youghiogheny River, often not more than a small creek, polluted immediately upstream by raw, municipal sewage from Oakland. The renovated water, studied in Phase 2 meets the criterion of being the most desirable, feasible raw water source, and will receive the additional full-scale, normal water treatment during the Phase 3 trial period of reuse.

Another relevant and similar statement on the nature of the water source may be found in Appendix A of the E.P.A. Interim Primary Drinking Water Regulations, which discusses the concepts and rationale used in their development (25):

> "Production of water that poses no threat to the consumer's health depends on continuous protection. Because of human frailties associated with protection, priority should be given to selection of the purest source. Polluted sources

> should not be used unless other sources are economically unavailable, and then only when personnel, equipment, and operating procedures can be depended on to purify and otherwise continuously protect the drinking water supply."

In addition to the criterion of best available source already addressed, this statement specifically focuses on economics and dependability. That this renovation system is indeed economical has been shown in a previous report (7). Sufficient data have been accumulated to demonstrate its dependability, and experience with such seasonal problems as freezing and overturns of the lagoons have indicated conditions when it should not be used to supplement the well water supply.

Nevertheless, one can and perhaps should put aside the legal question of potability and consider the following. If this renovated water were to be recycled into the plant as described and used as intended, would there be any significant, discernible risk to the consumers of the chickens processed there? It may be concluded, reasonably, after weighing all the results of this study and that which preceded it, that the Phase 3 trial period of full recycle and reuse, if instituted, would not jeopardize the public health. The basis for this judgement may be summarized as follows:

1) There are no apparent quantities of chemicals or micro-organisms in the renovated water harmful to human health, even if the water were directly consumed, which is not the intention.

2) The wastes generated within the chiller from the carcasses themselves, and to which subsequent ones are exposed, constitute a much greater source of contaminant exposure than does the renovated water.

3) The only possible likely source of exposure not identified that might cause concern is the category of chlorinated organics generated by disinfection of the poultry wastes. Yet chlorination of chiller water is commonly practiced and is not known to be hazardous to humans.

The question of using judgement rather than regulations to make a decision in this instance is probably the central issue. The federal agencies involved in the decision were rightly concerned about the possible precedent in approving this system for reuse. Aside from questions about specific chemicals that might be harmful, the probable underlying concern was that approval would be a precedent for the direct use of wastewaters for reuse before being able to fully develop criteria more stringent and elaborate than we now have for our natural waters used as the raw water source. Nevertheless, in the absence of such criteria careful judgement can and should allow Phase 3 to proceed. In view of the urgent need to conserve our water resources, limit waste discharges, and improve water quality, the nation will have to proceed to selective reuse of wastewater. Such a project as this is a useful step in that direction.

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APPENDIX A

MISCELLANEOUS METHODOLOGY

STATISTICAL

Statistical analyses were performed to compare sets of measurements for various parameters found in the water, wastewater, renovation system and carcass washings. The t-test parameter was used to determine if two sets of data came from the same population at given levels of confidence (21). Additionally, in some cases similar analyses were performed to assess which mean was greater after they were first determined to be different.

In using the t statistic the assumption is made that the tested parameters in the two groups are independent, normally distributed and have equal variances. However, for relatively small samples (less than 30, which usually was the case in this study) moderate departures from normality do not interfere with the validity of the analysis.

In most instances the parameters being compared had no temporal relationship to one another, even though the samples were taken on the same date. For example, the time of travel for the wastewater (A) to pass through the lagoons and appear as a renovated water sample (E) was about two weeks, the residence time in the lagoons. Thus, it is not useful to compare only those samples taken on a single day. For this reason principally, all comparisons were made on grouped rather than paired data.

In each comparison the hypothesis tested initially was whether the population means were identical. The level of confidence selected for this determination was 95 percent (a 5 percent level of significance, or alpha equal to 0.05). In testing this hypothesis a two-tail test was performed. In those cases where the hypothesis was also tested as to which population mean was larger, a one-tail test was used, also at alpha equal to 0.05.

The conclusions drawn from the analyses discussed above are described in the main body of this report in the following terms: when the t-test parameter indicates that the population means are identical at alpha equal to 0.05, it is concluded that the two sets of data for a given parameter, such as in the renovated or treated well water, are statistically identical. Similar language is used to refer to which of the groups have a larger population mean. For example, in Phase 2 for fluoride the two-tail t-test indicates that the raw wastewater (A) and renovated water (E), with respective means of 128 and 151 µg fluoride per liter, come from statistical indistinguishable populations at alpha 0.05. They are, however, different from the treated well water (Z) with a mean of 58 µg per liter. In addition, the

one-tail test confirms the fact that the means of A and E are larger than that of Z. It is reported then that for fluoride A = E > Z, and it is stated that the fluoride levels in A and E are statistically identical or equal, and greater than that of Z.

ORGANIC CHEMICAL ANALYSIS

Volatile Halogenated Organics

The methodology for the volatile halogenated organics in water samples was essentially an adaptation of that of Bellar and Lichtenberg, the purge and trap technique coupled with gas chromatography (2). A commercial purge and trap instrument was used, the Liquid Sample Concentrator, Model LSC-1 (Tekmar Company), along with a Packard 7400 Series gas chromatograph with a tritium electron capture detector.

Usually 5 ml samples were concentrated and analyzed, following their collection and sealing in serum vials. Both isothermal and temperature programming was utilized, the GC column being packed with Chromosorb 101 and the Liquid Sample Concentrator directly coupled to the gas chromatograph. The system was calibrated with known concentrations of volatile halogenated organics, such as chloroform, carbon tetrachloride, and methylene chloride, using both peak heights and areas of the chromatograms. The detection limit for chloroform, the only such compound detected by the GSPH laboratory, was approximately 1 µg/l.

Non-volatile Organics

The general procedure utilized in this study for the identification of non-volatile trace organics in water involved the extraction of these compounds from samples into a suitable solvent, followed by concentration of the extract into a volume acceptable for identification by GC-MS analysis. For the most part the techniques were similar to or adaptations of those described by Webb, Garrison, Keith, and McGuire (28).

All glassware was cleaned to remove trace organics, and samples were collected and handled so as to contact only glass or Teflon. Samples were typically 3000 ml and the pH was adjusted to 6 to 8 prior to extraction with high purity methylene chloride. Samples were extracted with three volumes of solvent, which were then subsequently combined. Sodium sulfate was used to absorb water from any emulsions that were formed, and to dry the extracts. Using a 3-ball Snyder column and Kuderna-Danish flask, the solvent was distilled from the extract at 72°C until about 1 ml of the latter remained. The volume was then further reduced to any desired level by directing a stream of clean, dry, inert gas over the surface of the tubes. The concentrated extracts were then injected into the GC or GC-MS for analysis. In some cases the concentrated extracts were first methylated using diazomethane (29). In all instances double-distilled water was analyzed also in the extraction and subsequent analysis as a control for possible contaminants introduced at any stage of the concentration or analytical procedure.

GC analyses were performed on a Packard Model 824 with flame ionization detector. Glass columns were utilized, usually with 3% OV-17 packing on Supelcoport 80-100 mesh and temperature programming. Similar or identical conditions were then used on the LKB 9000 GC-MS instrument, which was interfaced to a Digital Equipment Corporation PDP 11 computer to normalize the data and subtract background. Qualitative identifications (MS assignments) were made with the aid of the Registry of Mass Spectral Data by Stenhagen, Abrahanisson and McLafferty, but no concentration levels were established. The identifications must be regarded as presumptive, since they were not confirmed by direct comparison using known compounds.

MICROBIOLOGICAL AND RELATED METHODS

Residual Drug Assay

A disc assay method modified from the procedure of Huber et al. (15) was employed to detect residual drugs in the water and carcass samples. The first method used in this laboratory employed Bacillus subtilis (Difco spore suspension containing approximately 10^{10} spores/ml) as the test organism and seed agar (antibiotic medium no. 1) as the test substrate. On each day of testing, assay agar plates (15 x 100mm) containing 21 ml of base agar (antibiotic medium no. 2) were prepared. After solidification of the base agar, 4 ml of seed agar containing 10^8 Bacillus subtilis spores per ml were evenly poured on top of the base agar and allowed to solidify. Blank 0.5-inch filter paper discs (three discs per sample) were impregnated with the water and carcass samples and immediately placed on the assay plates. Each plate contained additionally one antibiotic control disc impregnated with 0.5 units of penicillin-G per ml prepared by diluting a vial of penicillin-G powder with double distilled water. After drying at room temperature, assay plates with the impregnated discs were inverted and incubated at 37°C for 12 to 16h. The sizes of the zones of inhibition on each plate were then measured.

This procedure was further modified according to the method of Read et al. (19) in order to detect bacitracin and enhance the sensitivity of detecting sulfa drugs and antibiotics in the water and carcass samples. Bacillus cereus and Mueller-Hinton agar were thus employed as the test organism and the test substrate, respectively. The control antibiotic discs were impregnated with 1 mg of streptomycin-sulfate per ml instead of penicillin-G. The assay agar plates were prepared as described previously with the above modifications.

To further increase the sensitivity Sarcina lutea was also employed as the test organism, since Read et al. (19) showed that this organism is more sensitive to bacitracin, which is used in the feed of the chickens.

Salmonella Methodology

Isolation and Identification--

Salmonellae were isolated from the water and carcass samples using standard Millipore membrane filtration (MF) techniques (1). Quantitation of Salmonella was attempted using three 10-fold dilutions, five tubes per dilution,

to determine the MPN index of organisms per 100 ml. The technique consisted of filtering five-aliquot volumes per sample through 47 mm diameter, 0.45μ pore-sized Millipore membrane filters (Millipore Corp., cat. no. HAWG047SO). Each filter was placed into 10 ml of enrichment broth to produce five tubes in the first row of the MPN test. Tubes were agitated for 2 to 3 min to remove organisms from the filters. Two 10-fold dilutions were made into enrichment broth to complete the 15 tubes for the MPN test.

In an attempt to improve the recovery of Salmonella during this study, either selenite brilliant green (SBG, Difco Laboratories) or tetrathionate (TET, Baltimore Biological Laboratories) broths were employed as enrichment media. After incubating the SBG tubes for 12 to 16h, or the TET tubes for 24h, both at 37°C, one loopful of enrichment broth from each tube was streaked on brilliant green agar (BGA, BBL) plates, with or without the addition of 0.08 g of sodium sulfadiazine (Lederle Lab. Div., Am. Cyanamid Co., Pearl River, N.Y.) per liter of agar. All plates were incubated at 37°C for 18 to 24h. The BGA plates were examined for pink-colored colonies, which were then subjected to the oxidase test (8). Oxidase-negative, pink-colored colonies were identified on the basis of their biochemical reactions in triple sugar iron (TSI) agar (BBL), lysine iron agar (LIA, BBL), motility indole-ornithine (MIO, Difco Laboratories) medium and an Enterotube (Roche Diagnostics). In all of the latter tests incubation was at 37°C for 18 to 24h. Organisms that produced typical Salmonella-like reactions (8) in TSI agar, LIA and MIO medium, and were identified as Salmonella by the Encise system (Roche Diagnostics), were inoculated into sugar fermentation tubes containing purple broth base medium (Difco Laboratories) and 1% of either raffinose, rhamnose or trehalose. After incubation at 37°C for 12 to 18h, the Salmonella isolates were speciated based on the sugars that were fermented (9).

Recovery Efficiency of MF Technique--

The recovery efficiency of the MF technique for S. typhimurium was determined as follows: five replicate 100-ml volumes from the Salmonella-inoculated distilled water sample were filtered. The filters were placed on trypticase soy agar (TSA, BBL) plates that were then incubated at 37°C for 18 to 24h. Colonies were counted for each filter and the mean number determined. The mean concentration of S. typhimurium per ml in dilutions of the TSB cultures (used to inoculate the distilled water) was calculated from the colony counts on 10 replicate TSA plates inoculated per dilution. The mean efficiency of recovery of the MF technique was 85%, calculated by comparing the number of colonies counted on the filters with those determined from the dilutions of the TSB cultures.

The MPN Test with Different Enrichment Broths--

Experiments were performed to determine the relative efficiencies of the SBG and TET enrichment broths, combined with BGA plates, for quantitating S. typhimurium by the MPN test. Also, single and mixed (with S. typhimurium) cultures of E. cloacae were similarly studied in the enrichment broths to determine the relative enrichment and inhibition of another organism commonly isolated from the water samples, and its possible influence on Salmonella quantitation. For the inoculated water samples MPN indices for S. typhimurium were significantly higher in the TET broth than in the SBG enrichment. MPN indices in TET were also higher (in single cultures) for S. typhimurium than

for E. cloacae, indicating that TET broth could both support the growth of Salmonella and repress that of Enterobacter. The results from these experiments suggest that Salmonella organisms may grow to higher concentrations in TET broth than in SBG enrichment, thereby facilitating quantitation by the MPN method.

Viral Methodology

Virus Analysis Using Chick Embryo Cells--

Primary chick embryo (CE) cell cultures were used with a plaque assay to detect the presence of avian viruses (e.g., NDV) in the water and carcass samples. CE cells were prepared from trypsinized embryos. The volume of cells was determined after centrifugation, and a 50% cell suspension was prepared in minimum essential medium supplemented with calf serum, sodium bicarbonate, antibiotics and mycostatin. CE cells were dispensed into tissue culture plates which were then incubated at 37°C in a 5% CO_2 atmosphere until complete cell monolayers developed.

Water and carcass washing samples were filtered through 0.45μ pore-size Millipore Swinnex filters to remove any bacteria which might have been present. Cell monolayers were inoculated with the undiluted and the 10^{-1} dilution of the samples. Negative and positive controls included uninoculated and NDV inoculated cell monolayers, respectively. After inoculation, plates were incubated for 1 hr. at 37°C. To each plate, agar overlay medium was added. Plates were reincubated at 37°C for 96 hr, then stained for plaques using a solution of neutral red. Plaques were counted and plaque forming units per ml (PFU/ml) were determined.

Presence of Avian Viruses in Tissue Extracts by Hemagglutination--

Spleens, livers and lungs were removed from chickens transported to the laboratory on ice from the processing plant. Using a modification of the procedure of Hofstad (13), 10% suspensions of the tissues were prepared in nutrient broth and centrifuged. The supernatant fluids, filtered through Millipore Swinnex filters to remove bacteria, were used to inoculate the allantoic cavity of embyronated eggs. Inoculated eggs were incubated at 37°C in a moist environment and candled daily. Eggs which showed dead embryos after 24 hr were chilled overnight at 4°C, after which allantoic fluids were harvested and pooled.

Hemagglutination tests were performed on the allantoic fluids by a modified procedure of McCollum and Brandly (18). Serial 2-fold dilutions of the fluids were prepared in saline, and each dilution was mixed equally with a suspension of chicken red blood cells. NDV and influenza-A viruses were included in the test as positive controls. The test was then read after 45 min of incubation at room temperature. The end point was the highest dilution of allantoic fluid that showed 1+ agglutination. The reciprocal of the highest dilution was the hemagglutination titer.

NDV Survival--

Experiments were designed to study the effect of temperature and light on the survival of NDV. Flasks of lagoon, filtered lagoon and distilled (included as controls) water were inoculated with NDV and placed either in

the light or the dark at 7 or 25°C. Flasks were gently agitated continuously during the experiments to simulate the aeration of the lagoon water at the poultry processing plant. Samples from each flask were removed at 0, 24, 48, 72, 96 and 120 hr post inoculation, then were inoculated onto primary CE cells and assayed for plaques as described previously. Plaques were enumerated and PFU/ml were determined.

APPENDIX B

CALCULATION OF STEADY-STATE IN RECYCLE

There is often concern about the possible build-up of waste constituents in water reuse and recycling. At first it might seem that such materials would continue to accumulate during each recycle until, perhaps, they would reach solubility limits. However, it can be shown readily that a steady-state concentration, rather than an infinite build-up, will eventually be attained, as long as some fraction of the parameter in question is lost in recycle, whether through removal in treatment or the discarding of a portion of the wastewater flow. In the latter case, the flow that is not utilized would be replaced by a "fresh" source of water.

In the proposed recycle system at the Sterling Plant, 50 percent of the lagoon effluent would be discarded to the river, and the remainder would undergo renovation, be mixed 50/50 with the untreated well water, and then the mixture would receive "normal" water treatment. For this system one can readily determine the maximum possible steady-state concentration, even when there is no reduction at any stage of treatment. For example, if a constituent were added to the wastewater (as a result of processing the poultry) at a rate of A grams per day, none of it was present in the well water, and none added or removed at any stage of treatment, one half of the A grams would still be lost to the receiving stream. In this case, it is obvious that the maximum (steady-state) quantity of the constituent that would "leave" the plant in the wastewater would be 2A grams per day. Half of this, or A grams, would be emitted to the river. The other half would be returned with the renovated water and added to the A grams generated at the plant, the maximum net of 2A grams per day thus appearing in the raw wastewater.

Where there is also partial removal at various stages of treatment, addition as a result of the use of chemicals in the treatment and renovation systems, or the constituent is also present in the well water, the steady-state calculation will be different from that resulting in the simple maximum factor of two shown above. It is useful to consider the effects of these factors and, at the same time, evaluate the rate at which steady-state will be achieved, i.e. the number of cycles required.

The following derivation for the steady-state equations in actual recycle is based upon the specific design of the Sterling plant, although the general principles can be applied to any reuse system. The derivation will refer to the flow diagram for the treatment plant and renovation system shown schematically in Figure B-1. In actual recycle the flows Z_f, C_f, and E_f, shown in Figure B-1, would each be 50% of that from the processing

plant. (In Phase 2 they were 100, 100 and 33%, respectively, of the flow from the processing plant, once steady-state was achieved.) It is assumed for simplicity that there is zero concentration of parameter A in the untreated well water, and that A grams per day of this parameter are added to the system from the processing of the poultry at the plant and emitted with its liquid waste effluent. In addition to the 50 percent loss of parameter A that occurs at the point where half of the chlorinated effluent from the second lagoon is discarded to the river, there can be prior fractional removal (1-b) in the two lagoons, as well as subsequent fractional removal in the renovation system (1-c), including that due to normal treatment of the mixture. It will be assumed that the fractional removal of parameter A in each case will be independent of its concentration. The time for a complete cycle (the period typically required for a waste constituent to be returned to the poultry plant in the renovated water) is determined essentially by the retention time in the lagoon system, approximately two weeks. Although one can expect continuous changes in concentration of a waste constituent as it proceeds through a cycle, it is convenient for the calculations to consider these after each such period.

At the start of the first cycle, the amount of waste parameter A that is emitted in the plant effluent is A grams per day, since no additional quantity has yet been added from the renovation system. At the end of the first cycle, this material now returns to the plant, but is reduced in quantity by the three factors discussed above: its loss to the receiving stream, reduction in the lagoons, and reduction in the renovation system. The net effect is that the quantity returned (per day) to the plant at the end of the first cycle is (A)(b)(c)/2. Thus, at the first day of the second cycle, the amount leaving the plant will be increased by this quantity, and will, therefore, be A + A(bc/2), or A(1 + bc/2).

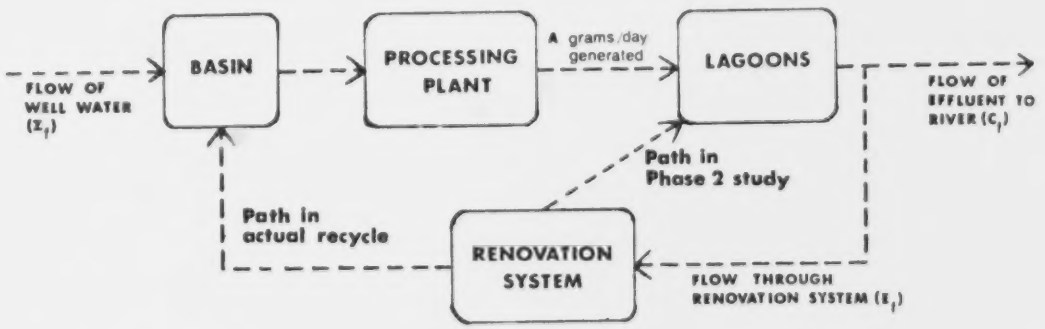

Figure B-1. Flow diagram for steady-state calculations.

Similarly, at the end of the second cycle, the amount of A that is returned to the plant will be $A(1 + bc/2)(bc/2)$. Again, on the first day of the second cycle this will be added to the A grams per day "generated" at the plant, so that $A + A(1 + bc/2)(bc/2)$ will leave the plant, this quantity being $A\{1 + (bc/2) + (bc/2)^2\}$. After "n" cycles this becomes:

$$L_n = A\{\sum_{n=0}^{n}(bc/2)^n\} \quad (1)$$

where L_n is the amount (or load) of parameter A in grams leaving the plant per day just after cycle "n". After an infinite number of cycles, and since bc/2 is less than unity, this series converges and the quantity becomes:

$$L_s = A/\{1 - (bc/2)\} \quad (2)$$

This then represents the amount of material, L_s, leaving the plant each day in the steady-state.

One can similarly determine the concentration of A reaching the plant in the renovated water in the steady-state, C_s (in effect is concentration after mixing 50/50 with the well water, the total flow being V liters per day through the plant):

$$C_s = (L_s - A)/V \quad (3)$$

since the daily load coming into the plant is less than that going out in the waste by A. With substitution of Equation 2 into 3:

$$C_s = [A/\{1 - (bc/2)\} - A]/V \quad (4)$$

which can be rewritten as:

$$C_s = (A/V)(bc)/(2 - bc) \quad (5)$$

Equations 1 through 5 are useful in assessing the possible build-up of concentration of a waste-parameter in a renovation system, and relating this to the pertinent parameter in the equation, namely the daily amount of waste generated, A, and its fractional removal in the lagoons and renovation process, (1-b) and (1-c), respectively. The equations can be used to estimate steady-state concentrations without actually going to full recycle. That is, if one can determine b and c removal parameters, as well as A, one can use Equation 5 to estimate the concentration of A in steady-state in actual recycle.

Let us now consider an example which approximates conditions in the Sterling plant study for total dissolved solids (TDS):

-Assume 820 mg/l TDS in processing plant waste effluent in first cycle.
-Assume 370 mg/l TDS in lagoon effluent in first cycle.
-Assume 335 mg/l TDS in renovation system effluent in first cycle.

-Thus, b = 370/820 = 0.45 (55% removal); c = 335/370 = 0.91 (9% removal; and bc = 0.41

Now one can calculate, using Equation 5, the expected steady-state concentration of TDS in the 50/50 mixture:

$$C_s = 820(0.41)/(2 - 0.41) \tag{6}$$

$$C_s = 211 \text{ mg/l TDS} \tag{7}$$

Before 50/50 mixing with the well water, the steady-state concentration would be twice this value, 422 mg/l, about 25 percent higher than that in the renovated water before 50/50 mixing at the end of the first cycle. As one can see from this example, the amount of build-up is not necessarily high and may be acceptable in many cases.

One can also estimate the number of cycles required to approach steady-state. This can be important in doing a study where the time available and, hence, the number of cycles is limited. One useful approach is to compare L_n and L_s. The ratio of the two is a measure of the difference between the daily waste emitted after n cycles and in the steady-state. On the first day after the first cycle this ratio becomes:

$$L_1/L_s = (1 + bc/2)(1 - bc/2) \tag{8}$$

Using the data for TSD from the example above,

$$L_1/L_s = (1.205)(0.795) = 0.96 \tag{9}$$

Thus, after only one cycle the daily load of waste emitted from the processing plant is 96 percent of that in the steady-state.

By the same token, one can also calculate the renovated water concentration in the steady-state compared to that at the end of the first cycle. One would obtain the same ratio for the renovated water either before or after mixing 50/50 with the well water and subsequent full scale water treatment, assuming the latter will have no effect on the parameter in question. (In fact, this assumption may be incorrect for many parameters. However, except for chemicals added in treatment, any additional effect would generally lower rather than raise the steady-state concentrations). In the general case, and using Equation 3:

$$C_n/C_s = (L_n - A)/(L_s - A) \tag{10}$$

At the end of the first cycle (n = 1), and using Equations 1 and 2, this becomes:

$$C_1/C_s = (1 - bc/2) \tag{11}$$

For our TDS example, this ratio is 0.795, which indicates that at the end of the first cycle the concentration of waste parameter A in the renovated water, either before or after mixing with the well water, will be 79.5 percent of that in the steady-state.

After two cycles, for the wastes leaving the plant:

$$L_2/L_s = \{1 + bc/2 + (bc/2)^2\}(1 - bc/2) \quad (12)$$

For the example at hand, this ratio is 0.991. The comparable ratio for the water is:

$$C_2/C_s = \{1 + bc/2\}(1 - bc/2) \quad (13)$$

which is equal to 0.958. Thus, in this case, after only two cycles the TDS concentrations in the wastewater effluent and the renovated water have reached 99 and 96 percent, respectively, of their steady-state concentrations. It can also be shown readily that, the higher the fractional removal (the smaller the factor bc), the faster will be the attainment of steady-state. After "n" cycles these ratios can be expressed as:

$$L_n/L_s = (1 - bc/2)\{\sum_{n=0}^{n}(bc/2)^n\} \quad (14)$$

$$C_n/C_s = (1 - bc/2)\{\sum_{n=0}^{n-1}(bc/2)^n\} \quad (15)$$

Another question of interest is whether the Phase 2 study can be expected reasonably to simulate the build-up of waste constituents in the renovation system that occurs in actual recycle. In Phase 2 the renovated water was diverted into the lagoon system, rather than being mixed 50/50 with the well water. These two paths are shown in Figure 1. Perhaps the most important difference is that in Phase 2 the flow through the lagoons was about 1/3 higher than it would be in actual recycle (the flow through the renovation system was about 1/3 of that through the plant, and both effluents were emitted to the first lagoon). This results in a higher dilution of any waste constituent emitted to the lagoon and cycled through the renovation system. Equations for the flow regime in actual recycle thus have to be modified to accurately reflect Phase 2. It can be shown readily that Equation 1 would then take the form:

$$L_n = A\{\sum_{n=0}^{n}(bc/4)^n\} \quad (16)$$

which becomes, in the steady-state,

$$L_s = A/\{1 - (bc/4)\} \quad (17)$$

The factor 4 appears because one fourth of the effluent from the lagoons is returned to the renovation system, the other three fourths being emitted to the river. Similarly, one can show that:

$$C_s = (L_s - A)/(V/3) \quad (18)$$

This is a direct analogy to Equation 3, but in this case the factor 3 appears because the daily flow through the renovation system is one third of that emitted from the poultry processing plant. With the insertion of Equation 17 into 18, the latter becomes:

$$C_s = (A)(3/V)(bc)/(4 - bc) \quad (19)$$

If one wishes now to compare the steady-state concentrations likely to be attained for the Phase 2 and full recycle systems, one would simply take the ratios of Equations 2 and 17 for the wastes, and 5 and 19 for the renovated waters. For the latter case, and designating r_s as the ratio of the steady-state concentrations in the renovated water in actual recycle to that in Phase 2, one obtains:

$$r_s = (1/3)(4 - bc)/(2 - bc) \quad (20)$$

Thus, this ratio is highly dependent on the fractional removals in the lagoon and renovation systems. With high degrees of removal (bc approaching zero) the ratio will approach 2/3. However, with very low removal (bc not much less than unity), the ratio will approach unity. Thus, one can estimate that the steady-state concentrations in actual recycle should be 0.67 to 1.0 times those observed in Phase 2.

There are, however, additional factors that should be considered. First, in actual recycle there can be additional removal of a waste parameter after the renovated water is mixed 50/50 with the well water and receives additional "normal" water treatment. Thus, the "c" term might be lower in actual recycle. Second, there was a higher flow through the lagoon system in Phase 2 compared to that which would occur in actual recycle. Thus, in the latter case with the lower flow, there would probably be greater degradation or loss for some waste constituents, and, again, "b" would be lower. For those parameters affected, the steady-state concentrations in actual recycle would be less than those predicted by Equation 20.

Another factor of possible importance is the role of the well water as a contributor of material to the renovation system. In the above derivations it was assumed that any waste constituent generated at the poultry plant was at zero concentration in the treated well water. One can modify this and divide the daily generated waste in Phase 2, A, into A' generated at the plant and A" contributed from the treated well water. Such a division has no bearing on the Phase 2 calculations, since they do not distinguish between these two possible sources of the waste parameter appearing in the plant effluent. However, in actual recycle, A would often be smaller than it would in Phase 2, since one source of it, A", would be reduced by half as a result of the 50 percent usage of well water compared to Phase 2 during which the well water flow was 100% of that of the effluent from the plant. For example, if in the Phase 2 study a constituent, such as magnesium, had a concentration in the treated well water 60 percent of that in the wastewater fron the plant,

then the Phase 2 value of A is made up of A', that generated at the plant, and A" = 0.6A', that generated in the well water. Thus, in Phase 2, A = 1.6A'. That is, the total amount of this parameter emitted from the plant is 60 percent higher than that generated at the plant, A'. However, with actual 50/50 recycle the well water would contribute only 0.3A', so that the sum of the magnesium from the latter and that generated at the plant is A' + 0.3A' = 1.3A', less than the 1.6A' emitted in the Phase 2 study.

The effect of this factor can be seen in the example given earlier for TDS (total dissolved solids). The additional piece of information needed is the TDS concentration in the treated well water, typically 155 mg/1. Thus, using the concepts above, the load in actual 50/50 recycle generated at the plant plus that due to the TDS in the renovated water would be 820 - (155/2) = 742 mg/1. This would directly reduce the steady-state concentration of TDS in the renovated 50/50 mixture from 211 mg/1 (from Equation 6 and 7) to 211(742/820), or 191 mg/1, about 10 percent less. The greater the relative concentration of a given parameter in the well water compared to the amount generated in the plant, the greater is the need to make this modification. In any event, it always operates in a direction to reduce the calculated steady-state concentration of a waste parameter in the renovated water delivered in actual recycle compared to that measured in Phase 2.

In considering all the factors, one can conclude that the Phase 2 study can be used to predict reasonably well the steady-state concentrations that can be expected in the 50/50 renovated water mixture in the actual recycle. It is also apparent that there are additional factors that could play a role and somewhat alter the conclusions. However, even in those circumstances additional measurements can facilitate the use of modified steady-state equations, and thereby compensate for them.